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THE PUNCTULATUS COMPLEX OF ANOPHELES (DIPTERA: CULICIDAE)

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INTRODUCTION

Anopheles punctulatus Dönitz and its close relatives have a range which extends from the Moluccas in the west, through New Guinea, the Bismarck Archipelago, and the Solomon Islands, to the New Hebrides in the east. One species is also widespread in northern Australia. With such an extensive distribution, which involves not only diverse environmental conditions but also the possibility of the development of strains or races as a result of isolation in some of the many islands, it is not surprising that a number of morphological differences have been observed among these mosquitoes, the significance of which has not been clear.

The type form was described by Dönitz (1901) from material collected by Koch at Stephansort, New Guinea. The adult female was shown to have a proboscis which was dark on the basal half, and pale on the apical half, with a small dark area just before the labella.

Laveran (1902) named as *A. farauti* a species from Efate, New Hebrides, the proboscis of which was "white at the extreme apex," thus implying that the labium

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was completely dark. The variety *moluccensis* with a completely dark labium and pale labella was next described by Swellengrebel and Swellengrebel de Graaf (1920a and b) from material collected in a number of localities in the Moluccas. Although they emphasize the separation of *moluccensis* from *punctulatus* Dönitz by the *completely dark labium* of the former (1920c), the Dutch workers soon began to include with *moluccensis,* specimens with a small ventral white patch on the apical third of an otherwise completely dark labium. Swellengrebel and Swellengrebel de Graaf (1920a and b) show that the larva of *moluccensis* differs from that of *punctulatus* Dönitz especially in the structure of the anterior submedian hairs 1 and 2, which have thick stems and arise from heavy, fused tubercles in the former, while these hairs are slender and have small, widely spaced tubercles in the latter. Thus, when de Rook (1924) isolated larvae with *moluccensis* characters, and reared from them females with a small ventral white patch near the apex of the proboscis, he concluded that *moluccensis* females could have either a completely dark labium or one with the ventral white patch. From Swellengrebel and Rodenwaldt (1932), one learns that females with the dark labium must be relatively rare in the Netherlands Indies, for in their description of *moluccensis* they state that only 4 per cent had this character, while 96 per cent had the ventral white patch.

Meanwhile, the relationship between *punctulatus* Dönitz, *moluccensis,* and other species was being clarified. Theobald (1910) thought that *A. tesselatus* Theobald was synonymous with *punctulatus* Dönitz, while Swellengrebel and Swellengrebel de Graaf (1920b) listed *tesselatus* as a variety of *punctulatus.* At Pionierbivak, de Rook (1924) noted that larvae from which he was rearing typical *punctulatus* adults were different from larvae of *"N. punctulata"* (= *tesselatus*) from Malaya, Sumatra and Java, thus proving that the latter was a distinct species, to which the name *tesselatus* should be applied. Swellengrebel and Swellengrebel de Graaf (1920a) described *moluccensis* as a variety of *annulipes,* but Edwards (1921) recognized its closer affinity to *punctulatus* and listed it as a variety of that species. In fact, Edwards even doubted that *moluccensis* was distinct from *punctulatus.* His study of material in the British Museum failed to reveal a single individual which agreed with Dönitz's description of the apically pale proboscis; instead, all of the specimens had completely dark probosces, except one or two, which had "a pale area on the underside of the proboscis towards the tip." One of these specimens had been collected at Stephansort, the type locality of *punctulatus,* and since Dönitz himself had identified it (presumably as *punctulatus*), Edwards felt that Dönitz's original description was based upon an abnormally pale individual of the same species that Swellengrebel and Swellengrebel de Graaf subsequently described as var. *moluccensis.* However, he provisionally recognized two varieties: *punctulatus,* with the apical half of the proboscis pale, and *moluccensis,* which he defined as having either a completely black proboscis or one with a small ventral pale area towards the tip. Even so, he points out that the latter variety "is *A. punctulatus* in the sense in which I have previously understood it," which interpretation may have led him in 1924 to reverse completely the concept of the varieties, for at this time he considered *moluccensis* to have either a proboscis with a subapical ventral white patch or one completely white on the apical half, whereas in *punctulatus* the proboscis was completely dark. The records and identifications of *A. punctulatus* from the Solomon Islands and the New Hebrides, published by Buxton and Hopkins (1927), Paine and Edwards (1929),

and Senevet (1931b) are perhaps the result of this interpretation. In his 1932 monograph, from the inclusion of references to Buxton and Hopkins (1927) and Paine and Edwards (1929) under *punctulatus,* as well as the listing of *farauti* Laveran as a synonym of this mosquito, it would seem that Edwards still held this opinion at this date.

The only form which has been found in Australia (other than one doubtful record of *punctulatus* from Booroloola by Hill, 1925) and the New Hebrides has a completely dark labium. Thus in some Australian keys and descriptions (Taylor, 1929, 1943; Russell, Rozeboom and Stone, 1943), one sees no reference to the pale patch on the proboscis. But the intensive collections by military and civilian entomologists in the Australasian region have resulted in the finding of mosquitoes with the pale patch in a number of localities in New Guinea and even in Guadalcanal in the Solomon Islands. This rediscovery has led some investigators to consider the form, either consciously or unconsciously, as a third variety. While Lee and Woodhill (1944) do not recognize it as a true variety, they do not include it with *moluccensis,* and refer to it as an "intermediate form." They say, "Forms intermediate between these two subspecies (*punctulatus moluccensis* and *punctulatus punctulatus*) occur abundantly in some localities and these may show variable degrees of pale scaling on the proboscis and therefore cannot be referred to either subspecies on adult characters." Owen (1945) who discovered this mosquito on Guadalcanal, described it as a new species and named it *A. koliensis.*

Knight and Farner (1944) have shown that since Edwards' synonymy of *farauti* Laveran with *punctulatus* Dönitz is not valid, the first name applied to the black-proboscis variety was *farauti* Laveran. Thus the black-proboscis *moluccensis* described by Swellengrebel and Swellengrebel de Graaf becomes a synonym of *farauti;* furthermore, if the Dutch workers are correct in considering that the "intermediate form" is not systematically distinct from the dark-proboscis variety, the name *farauti* will have to be applied to this mosquito as well. Lee and Woodhill (1944), however, considered it possible that *farauti* may be distinct from *moluccensis,* for whereas the latter has heavily frayed or branched outer clypeal hairs, half or more the length of the inner clypeals, Buxton and Hopkins (1927) describe larvae from the New Hebrides with minute, simple outer clypeal hairs.

Before the systematics of the *punctulatus* complex can be clarified, the following questions must be answered:

1. Does *A. farauti* Laveran occur westward from the New Hebrides through the Solomon Islands, Bismarck Archipelago, and New Guinea to the Moluccas?

2. Is the dark-proboscis *moluccensis* of Swellengrebel and Swellengrebel de Graaf the same as *farauti* Laveran?

3. Do the "intermediate form" and the dark-proboscis form represent a single systematic entity, or are they distinct enough to merit subspecific or even specific rank?

4. Is *A. koliensis* of Guadalcanal the same mosquito as the "intermediate form" of New Guinea?

The purpose of this investigation was to accumulate morphological and experimental data that would answer the above questions. Specifically the objectives were as follows:

1. To study thoroughly the morphology of larvae, pupae, and adult females of

the members of the *punctulatus* complex, in order to find any possible additional differential characters.

2. To obtain material in sufficient quantities from several widely spaced localities within the range of the *punctulatus* complex, so that comparisons could be made between *farauti* of the New Hebrides and the dark-proboscis mosquito of Guadalcanal, New Guinea, and the Moluccas; and between *koliensis* of Guadalcanal and the "intermediate form" of New Guinea. It is of interest also to compare the typical *punctulatus* of Guadalcanal with this form in New Guinea, although the former agreed well with the descriptions of the type.

3. To determine whether the "intermediate form" and *koliensis* are systematically different from the dark-proboscis mosquito, and hence worthy of subspecific or specific rank.

PROCEDURES

Collection of specimens.—The occupation of certain areas by Japanese forces, as well as limitation in time, prevented collections in all but four localities in the *punctulatus* range. Rather than spending a short time in many places, which would have permitted only a small amount of material from each and no reared progeny, it was felt that a more satisfactory procedure would be to collect intensively in a few widely separated localities. Those selected were Espiritu Santo in the New Hebrides, Guadalcanal in the Solomon Islands, Hollandia in Dutch New Guinea, and Morotai in the Moluccas.

To make certain that the morphological differences in the adult females could be correlated with differences in the larvae and pupae, almost all of the specimens included in this study had larval and pupal skins associated with the pinned adult. The exceptions were some wild *farauti* larvae and pupae from Espiritu Santo, where this was the only species present, and a few *farauti* larvae and pupae from an area in Guadalcanal where intensive collections by Army and Navy malaria survey teams as well as by the authors showed that this was the only species present. Fourth-stage larvae, collected from natural breeding places, were isolated in individual containers; and after pupation and emergence of the adult, the larval and pupal skins were mounted in balsam on a slide and given the same accession number as that of the adult.

When an anopheline pupates, the abdominal segments of the cast larval skin are telescoped together. If mounted in this condition, most of the abdominal hairs cannot be examined with certainty, for they are obscured by the overlapping palmate hairs, and their relative positions are distorted. The skin must be stretched, but if this stretching is attempted after the specimen has been placed in balsam, the skin will be too brittle for satisfactory results. It was found that the best procedure was to stretch the skin in water as soon as possible after pupation. It was placed in a drop of water in a hollow-ground slide, and with the aid of a dissecting microscope and a pair of needles, the abdominal segments were gently drawn out. It was stored in a vial of alcohol until the adult emerged from the pupa; then the larval and pupal skins were run through 70 and 95 per cent alcohol and eugenol in preparation for mounting.

The pupal stage is best studied from the cast skin, which is firm enough to mount in balsam and still retain its complete shape. However, for proper examina-

tion, the skin should first be dissected. This dissection is best done in the balsam by inserting a needle between the junction of the metanotum and the cephalothorax proper and separating these two structures. Such a procedure automatically leaves the cephalothorax open along its dorso-median line and it can then be easily spread out and turned over for the permanent mount. The combined metanotum and abdomen is mounted as one piece. Edwards (1941) recommends dissecting the cephalothorax into three pieces by detaching each wing shield from the distinct central section that bears the head shield (vertical plate of Crawford, 1938), internal tentorial rods, proboscis and antennae.

As pointed out above, the Dutch workers obtained two kinds of adults from *moluccensis* larvae, and so it was evident that wild-caught material alone, even though the stages were associated, would not suffice. It was essential that the constancy of the differential characters be studied within progeny from individual females. For example, if the small white ventral patch is a simple color variant of the completely dark proboscis, one would expect to find both types of proboscis among females reared from a single batch of eggs. To obtain such progeny, blooded females were caught in native dwellings, isolated over strips of moist filter paper in test tubes, and permitted to lay eggs. Each egg batch was placed in a separate bowl, and the larvae were reared to the fourth stage. With proper care, most of the larvae matured without appreciable loss of hairs. First-stage larvae were fed yeast, and older larvae were given a powdered food consisting of a mixture of C ration biscuit, yeast and vitamin concentrates. Before pupation, these larvae also were isolated, so that even within the progeny from one female the individuals would have associated larval, pupal, and adult stages. However, a few larvae and pupae were included in the study whose adults were either males or had been lost, and many adults were included for which the larval or pupal skins were not available, or because lack of time prevented the study of as many larvae and pupae as of the adult females. This was permissible because these specimens were members of known progeny.[1]

Morphological studies.—It would have been impossible to make a detailed study of all structures, color patterns and complete larval and pupal chaetotaxy, of all the specimens in the large collections which were obtained. Consequently, an intensive preliminary morphological study was made of a relatively few individuals of the dark-proboscis *farauti* from Espiritu Santo and Guadalcanal, and of *koliensis* and *punctulatus* from Guadalcanal. This permitted the selection of characters which promised to be of use in differentiation, and they alone were examined in the study of the large amount of material.

The identity of each series was dependent upon the characteristics of the parent female. Records of the differential structures were kept separately for each series, so that the degree of variation of offspring from parents could be determined with accuracy.

The specimens that were collected as larvae from natural breeding places were also identified by female characters. After the adult females had been placed into three categories, i.e., those with labium dark, labium one-third to one-half white apically, or labium with the small subapical white patch, associated larval and pupal skins were divided into corresponding groups and their differential characteristics tabulated.

[1] For convenience, progeny reared from individual females are hereafter referred to as "series."

RESULTS

Differential characters of adult females.—The separation of the adults of the *punctulatus* complex is based entirely upon the color pattern of the proboscis. Three basic types can be recognized:

Type A (Fig. 20). Labium completely dark-scaled, except for a very narrow ring of pale scales at the extreme apex, just basal to the yellowish labella. This ring is found in the other types as well, and has no significance. Rarely there are a few pale scales ventrally or laterally on the basal half of the proboscis; they also occur in the other types, and so were not considered. If only two or three pale scales were seen on the apical half of the proboscis, the specimen was considered to belong to type C, described below.

Type B (Figs. 21 and 22). Apical half of the labium pale-scaled, with a sharp line of demarcation between the black and white areas. A narrow, subapical black ring may occur just before the labella, but in many specimens the black ring is absent, so that the apical third or half of the labium, as well as the labella, is entirely pale. Some dark scales may appear within the pale area, but as they follow no pattern, they were not considered.

Type C. This is interdmediate between types A and B. Essentially it consists of a dark labium with a small patch of white scales on the underside near the apex (Fig. 23). However, the pale markings may exhibit considerable irregularities. The pale scales may number only two or three, so that there is only a tiny white patch, or there may be two or more such small patches (Fig. 24). Sometimes there may be a few scattered pale scales. In the other extreme, the pale markings become more extensive. Not only may the ventral area become large, but near its distal end pale scales may form a complete ring around the proboscis (Fig. 25). This dorsal marking may also become broad, and the only way to separate this pattern from that of type B is the difference in the width between the dorsal and ventral pale area (Fig. 26). A few specimens will show a broad pale area on the dorsal surface of the proboscis, while that on the ventral surface is smaller (Fig. 27). In these extremes of pale scaling, the subapical dark ring is still broad, and the line of demarcation between the pale and dark areas may be quite irregular. In this study, the arbitrary differentiation between the pale extremes of type C and type B depended upon the extent and regularity of the pale area on all sides of the proboscis. Of the 633 type C probosces examined (Table 1), the pale extremes constituted 9.9 per cent of the total; the rest had the small ventral pale patch or a few ventral pale scales.

The distribution of the proboscis types among the adult females is given in Table 1. On the basis of the proboscis, three distinct kinds of adults were recognized. Those with type B proboscis agreed with Dönitz's description, and were called *punctulatus*. All of the 269 *punctulatus* examined had a third to a half of the proboscis pale apically. Following the synonymy of Knight and Farner (1944) the mosquito with the completely dark proboscis was called *farauti*. Of the 707 series specimens from Espiritu Santo and Guadalcanal, only one was found with several pale scales on the apical half of the labium, and this proboscis was not a true type C, for the white scales were lateral rather than ventral. Those with type C proboscis from Guadalcanal were called *koliensis* in accordance with Owen's (1945) description. Data presented below will show that the "intermediate form" of New

Guinea cannot be separated from *koliensis* of Guadalcanal, and so this mosquito in Hollandia was also designated as *koliensis*. The identity of the few individuals of *koliensis* with type A or type B proboscis was certain, for they belonged to reared progeny.

Table 1 shows that of the 291 series individuals of *koliensis* from Guadalcanal, nine had type A proboscis. It is of interest to note that six of these nine belonged to a series of nine offspring produced by a single *koliensis;* the other three daughters had a type C proboscis similar to that of the parent. Since only once in a total of 47 *koliensis* series did an appreciable number of progeny have probosces unlike that of the parent, it seems possible that the abnormal series represents hybridization between *koliensis* and *farauti*. If eliminated from Table 1 on these grounds, it will be seen that of the 584 series specimens of *koliensis,* only six, or 1.0 per cent had a dark proboscis. These were scattered through four series. If the abnormal series

TABLE 1.—*Adult: proboscis*

Species	Locality	Source	Number examined	Distribution according to proboscis types		
				A	B	C
punctulatus	Guadalcanal	Wild	20	..	20	..
		9 series	143	..	143	..
	Hollandia	Wild	100	..	100	..
		1 series	6	..	6	..
	Total	.....	269	..	269	..
koliensis	Guadalcanal	28 series	291	9	7	275
	Hollandia	Wild	69	..	..	69
		19 series	293	3	2	288
	Total	.....	653	12	9	632
farauti	Espiritu Santo	Wild	12	12	..	..
		24 series	311	310	..	1*
	Guadalcanal	Wild	25	25	..	..
		24 series	396	396	..	..
	Hollandia	Wild	94	94	..	..
	Morotai	Wild	29	29	..	..
	Total	.....	867	866	..	1

* Not exactly a true type C proboscis, as there were a few white scales laterally on the apical third of the proboscis.

is not eliminated, the number of type A proboscis among *koliensis* progeny is raised to 2.1 per cent. The nine type B *koliensis,* which were scattered through six series, represent 1.5 per cent of the total series specimens. Thus, 96.4 per cent of the *koliensis* series individuals had type C proboscis.

Although none of the other characters of the adults was found to be reliable, some of them furnished supplemental means of differentiation, and so the ornamentation of the palpi, certain wing spots, and the markings of the tarsi were included in the study of the adults.

The basic coloration pattern of the palpus is illustrated in Fig. 28. There are four pale bands on the three distal segments; the fourth and fifth segments are broadly white apically, while the third segment has a narrow apical ring and a wide subapical white area which may be in the form of a complete band or a white spot on the dorsal surface only. The modifications of this pattern occurred in the pale markings of the third segment, on the basis of which the palpi were grouped into three types:

Type A (Figs. 28 and 29), with a broad white subapical band or dorsal spot on the third segment. Usually the apical pale ring is also distinct, but it often is reduced to a few pale scales.

Type B (Figs. 30, 31, 32 and 33), in which the subapical white area is reduced to a few pale scales, or is completely absent. The apical pale ring may be distinct, or may be reduced to a few scales or is entirely absent. The extreme of this palpus has no pale scales at all on the third segment.

Type C (Fig. 34), in which the subapical pale area is very large, and coalesces with the apical pale ring, at least on the dorsal surface. Each of the three distal palpal segments thus possesses a broad apical pale band.

The distribution of these palpi types is summarized in percentages in Table 2, which shows that palpi with three broad pale bands were common in *punctulatus,* whereas they were seldom seen in *koliensis* and *farauti.* There was a tendency for *koliensis* to have darker palpi; however, in 61.4 per cent of the *farauti* from Morotai, there also was a reduction of the white markings on segment III. The only value of the palpus in identification is the greater percentage of type C in *punctulatus,* which in some instances might be of use as a supplemental character in separating *punctulatus* from a *koliensis* with an exceptionally white-marked proboscis.

TABLE 2.—*Adult: palpi*

Species	Locality	Number examined	Distribution according to palp type		
			% A	% B	% C
punctulatus	Guadalcanal	199	87.4	...	12.6
	Hollandia	208	55.3	3.8	40.9
	Total	407	71.0	2.0	27.0
koliensis	Guadalcanal	300	62.4	35.3	2.3
	Hollandia	707	65.1	33.5	1.4
	Total	1007	64.2	34.1	1.7
farauti	Espiritu Santo	265	86.0	14.0	...
	Guadalcanal	361	71.7	28.3	...
	Hollandia	184	79.4	19.0	1.6
	Morotai	57	38.6	61.4	...
	Total	867	75.5	24.1	0.4

The wing of *punctulatus* has three small pale spots at the extreme base of the costa. Reduction of these humeral spots occurred most frequently in the adults with type C proboscis. Thus, of 759 *koliensis* wings, 11.1 per cent had one, 41.5 per cent had two, and 47.4 per cent had three humeral spots. Of 84 *punctulatus* wings, 98.8 per cent had three, and 0.2 per cent had two spots. Of 606 *farauti* wings, 4.0 per cent had two, and 96.0 per cent had three spots. The presence of only one or two humeral spots might be of use in distinguishing a *koliensis* with a pale proboscis from *punctulatus.*

The *punctulatus* wing illustrated by Ross and Roberts (1943) shows two pale areas on the costa slightly basad of the middle of the wing, between the third humeral and the subcostal pale spots. These are the sectoral and accessory sectoral spots, sometimes referred to as median spots. Ordinarily they are separated by the small sectoral dark spot, but in some of the wings they are fused into a single broad white area, and in this case only a single, broad, median, pale area is seen on the costa; nevertheless, both spots are present and in Table 3 they are included under the heading of two median spots. In *koliensis,* the accessory sectoral spot usually is absent, leaving only the narrow sectoral white spot, and in Table 3 this condition is referred to as "one median spot." Table 3 shows that all but 8.7 per cent of the wings of *punctulatus* had both sectoral and accessory sectoral spots, while in only

TABLE 3.—*Adult: median wing spots*

Species	Locality	Number wings examined	% with no median spot	% with 1 median spot	% with 2 median spots	
					Fused	Separate
punctulatus	Guadalcanal	323	..	6.2	2.8	91.0
	Hollandia	205	..	12.7	15.6	71.7
	Total	528	..	8.7	7.8	83.5
koliensis	Guadalcanal	467	..	94.4	1.9	3.7
	Hollandia	707	0.3	98.4	1.0	0.3
	Total	1174	0.2	96.8	1.4	1.6
farauti	Espiritu Santo	615	..	12.5	2.4	85.1
	Guadalcanal	782	..	6.9	11.1	82.0
	Hollandia	183	..	41.5	8.2	50.3
	Morotai	58	..	19.0	8.6	72.4
	Total	1638	..	13.4	7.4	79.2

3.0 per cent of the *koliensis* wings were both spots present. This character should be of use in separating *punctulatus* from a *koliensis* with a broadly white proboscis. Most of the *farauti* wings had two median spots, but in Hollandia only one spot was seen in 41.5 per cent of the wings, which limits the usefulness of this character as a means of distinction between this species and *koliensis*.

Records were kept of the dorsal markings of the tarsi of 100 or more fore, mid, and hind legs of *punctulatus* and *farauti,* and of over 300 *koliensis* legs, but the markings were not found to be of use. The tarsi of *punctulatus* had the most extensive white markings, while those of *koliensis* were the darkest.

Differential characters of the larvae.—In the search for morphological differences, complete studies were made of the chaetotaxy of 41 *farauti,* 21 *koliensis,* and 21 *punctulatus* larvae. In addition, other characters examined were the degree of pigmentation of the head and antennae, the structure of the spiracular apparatus, and the arrangement of the pecten spines. The external structure of a *farauti* larva from Espiritu Santo is illustrated in Figs. 1–4.

The pigmentation of the head and antennae has been used by various authors to separate *moluccensis* from *punctulatus,* and although in *punctulatus* larvae it is much lighter than in *farauti,* so many *farauti* larvae from natural breeding places had pale heads and antennae, while many *punctulatus* larvae had very dark heads and antennae, that the character was dropped. In *punctulatus* the pecten spines are often subequal in length, whereas in *farauti* and *koliensis* there usually is a marked difference between the longest and shortest teeth. A specimen with an even row of pecten spines may be identified as *punctulatus,* but one with uneven spines could be *punctulatus* as well as *farauti* or *koliensis*. Walch and Soesilo (1929) state that the pecten of *moluccensis* could not be distinguished from that of *punctulatus*. The pecten was not included in the statistical study.

According to published descriptions and keys, the outer clypeal hairs of *punctulatus* are half or less the length of the inner, while in *moluccensis* they are over half as long as the inner, and both inner and outer hairs are branched. This branching

TABLE 4.—*Larva: relative length of anterior clypeal hairs*

Species	Number examined	Outer/inner: distribution in percentages						
		0.3	0.4	0.5	0.6	0.7	0.8	0.9
punctulatus	173	1.2	30.6	63.0	4.6	0.6	..	..
koliensis	465	..	15.1	43.7	37.8	3.0	0.2	0.2
farauti	260	0.4	9.2	30.0	35.4	17.3	5.8	1.9

usually consists of fine lateral spicules along the shaft, but in *farauti* especially the outer clypeals may have a number of long, heavy branches near the tip. The results of the clypeal hair measurements, expressed in ratios of the outer to the inner, are presented in Table 4. In determining the ratios, the outer clypeal was always paired with the inner on the same side. Since the source of material or locality had little influence on the length of the hairs, the results are tabulated only according to species. From the table it will be seen that most of the outer clypeals of *punctulatus* larvae are half or less the length of the inner clypeals, while in *koliensis* and *farauti* they usually are half or more the length of the inner hairs. Since so many of the outer clypeals in all three species were about half as long as the inner hairs, they are of little value as a differential character.

Because it was observed that branching of the clypeal hairs was often more distinct in larvae taken from natural breeding places, the results of Table 5 are tabulated according to locality and also to source of material; that is, wild larvae

TABLE 5.—*Larva: branching of anterior clypeal hairs*

Species	Locality	Source	Outer clypeals			Inner clypeals		
			Number examined	Number branched	Number simple	Number examined	Number branched	Number simple
punctulatus	Guadalcanal	Wild	36	...	36	52	9	43
		7 series	87	...	87	205	26	179
	Hollandia	Wild	58	3	55	118	27	91
		1 series	3	...	3	7	2	5
	Total	.....	184	3	181	382	64	318
koliensis	Guadalcanal	15 series	170	31	139	197	136	61
	Hollandia	Wild	73	51	22	98	91	7
		19 series	356	51	305	478	374	104
	Total	.....	599	133	466	773	601	172
farauti	Espiritu Santo	Wild	47	39	8	40	37	3
		19 series	145	49	96	340	300	40
	Guadalcanal	Wild	66	66	...	63	41	22
		8 series	58	16	42	146	42	104
	Hollandia	Wild	100	87	13	108	100	8
	Morotai	Wild	37	36	1	47	40	7
	Total	.....	453	293	160	744	560	184

taken from natural breeding places, and larvae reared from eggs in the laboratory. Spicules were rarely found on the outer clypeals of *punctulatus,* but 16.8 per cent of the inner clypeals had these small branches, although usually they were difficult to find. Branching occurred in 22.2 per cent of the outer clypeals, and in 77.7 per cent of the inner clypeals of *koliensis.* This reversal in percentages of branching of outer and inner hairs may be due in part to the difficulty of distinguishing the minute spicules in the outer hairs, which are obscured to a large extent by the underlying mouth brushes. In *farauti,* 64.7 per cent of the outer clypeals were branched, often heavily so, and 75.3 per cent of the inner clypeals were frayed.

The environment seemed to have an effect not only on pigmentation of the head but also on branching of the clypeals. In wild *koliensis* larvae, 69.9 per cent of the 73 outer clypeals examined were frayed, while in the series larvae, of 526 outer clypeals, only 15.6 per cent were branched. A higher percentage of the inner clypeals of the wild *koliensis* larvae were also branched. In *farauti* from Espiritu Santo, branching occurred in 83.0 per cent of the outer clypeals of the wild-caught larvae, and in 33.8 per cent of the laboratory-reared series larvae. In Guadalcanal, 100.0 per cent of the outer clypeals of wild larvae, but only 27.6 per cent of these hairs in

series larvae, were branched. The above percentages do not emphasize sufficiently the differences in clypeal branching between wild and series larvae. In the former, the branches were long and heavy, and could be seen easily in spite of the underlying mouth brushes.

A larva with simple anterior clypeals, the outer less than half the length of the inner, is most apt to be *punctulatus,* whereas one with heavily branched outer clypeals, over half the length of the inner hairs, is *farauti* or *koliensis.* No heavily branched outer clypeals were seen among *koliensis* from Guadalcanal, perhaps because all of the larvae were reared in the laboratory.

Preliminary studies of the posterior clypeal hairs of the larvae in Guadalcanal indicated that the length of these hairs might be of considerable value in separating *koliensis* from *punctulatus* and *farauti,* for in the former they were very short, so that all but a few were not long enough to reach the tubercles of the anterior clypeals, whereas in the latter two species the posterior clypeals extended to or beyond the anterior tubercles. The source of the larvae appeared to have no effect on the length of the hairs, and so in Table 6 the data are tabulated according to species and

TABLE 6.—*Larva: posterior clypeal hairs*

Species	Locality	Number examined	Per cent reaching to or beyond anterior tubercles	Per cent not reaching anterior tubercles
punctulatus	Guadalcanal	230	90.4	9.6
	Hollandia	147	89.1	10.9
	Total	377	89.9	10.1
koliensis	Guadalcanal	191	5.8	94.2
	Hollandia	643	1.9	98.1
	Total	834	2.8	97.2
farauti	Espiritu Santo	257	99.2	0.8
	Guadalcanal	182	92.3	7.7
	Hollandia	131	19.8	80.2
	Morotai	45	42.2	57.8
	Total	615	76.1	23.9

locality only. This table shows that the posterior clypeals are usually long in *punctulatus,* short in *koliensis,* and that in *farauti* they are long in larvae from Espiritu Santo and Guadalcanal, but usually short in larvae from Hollandia and Morotai. Although the length of the hairs is a good character for distinguishing between *farauti* and *koliensis* of Guadalcanal, it is of no use in the western part of the range.

The number of branches of the occipitals were essentially alike in larvae from natural breeding places and in those of the laboratory-reared progeny; therefore the results in Tables 7 and 8, which show the distribution of the hairs according to the number of branches, are tabulated by species and locality only. The branching of the outer occipitals is a good means of differentiating *punctulatus* from *koliensis.* In the former, 93.4 per cent of the hairs were one- to three-branched, whereas in the latter 96.4 per cent had four or more branches. Since 97.5 per cent of the inner occipitals of *punctulatus* were single, and 98.3 per cent of these hairs in *koliensis* were two- to five-branched, they also serve as a differential character between these mosquitoes. Neither the inner nor the outer occipitals can be employed to distinguish between *farauti* and *koliensis;* however, the heavier branching of the occipitals in *koliensis* is additional evidence of a difference between these mosquitoes, even though it is of no use as a key character.

TABLE 7.—*Larva: branching of outer occipital hairs*

Species	Locality	Number examined	Distribution according to number of branches								
			1	2	3	4	5	6	7	8	9
punctulatus	Guadalcanal	257	36	156	59	6	..	..	..	..	..
	Hollandia	152	10	53	68	19	2	..	..	..	..
	Total	409	46	209	127	25	2	..	..	..	..
koliensis	Guadalcanal	189	..	..	4	15	49	82	23	13	3
	Hollandia	580	4	2	18	194	256	95	11	..	..
	Total	769	4	2	22	209	305	177	34	13	3
farauti	Espiritu Santo	393	21	168	162	42	..	..	..	..	..
	Guadalcanal	178	..	49	67	47	14	1	..	..	..
	Hollandia	127	..	6	36	52	32	1	..	..	..
	Morotai	43	..	..	7	16	19	1	..	..	..
	Total	741	21	223	272	157	65	3	..	..	..

The most certain character for the identification of *punctulatus* larvae is the structure of the anterior submedian thoracic hairs. As illustrated by Swellengrebel and Rodenwaldt (1932), prothoracic hair 1 in this species is very slender, whereas in *koliensis* and *farauti* it has a heavy, swollen stem. Furthermore, the tubercles of hairs 1 and 2 in *punctulatus* are very small, sometimes hardly more than a ring, and they are widely separated, while in *koliensis* and *farauti* they are large and generally fused together. The pigmentation of these hairs and tubercles was heavier in larvae from natural breeding places, but as the structure of the prothoracic submedian hairs was essentially alike in laboratory-reared and wild-caught larvae, the data in Table 9 are tabulated only according to species and locality. This table summarizes, in percentages, the number of slender and swollen inner submedian hairs (hair 1), and the number of fused or separate tubercles. Slender, lightly pigmented prothoracic submedian hairs, arising from well separated tubercles, are characteristic of *punctulatus* (Fig. 7). Although the tubercles of 74.2 per cent of the *koliensis* hairs in Guadalcanal were also separate, they were larger and more closely approximated than those of *punctulatus* (Fig. 6). The Hollandia *koliensis* larvae were similar to *farauti* in having large, fused tubercles and swollen innner submedian hairs (Fig. 5); thus the prothoracic group is of no use in separating these species. As in the case of *koliensis,* those *farauti* larvae which had separate tubercles could usually be separated from *punctulatus* by the larger size and closer proximity of the tubercles, as well as by the swollen hair 1.

Preliminary studies of the larvae from Guadalcanal indicated that the first abdominal palmate hairs might be of prime importance in identifying *farauti.* Table 10 summarizes the number of hairs with hair-like, slender, or broad, notched leaflets

TABLE 8.—*Larva: inner occipital hairs*

Species	Locality	Number examined	Distribution according to number of branches				
			1	2	3	4	5
punctulatus	Guadalcanal	253	248	5	...	...	..
	Hollandia	148	143	5	...	...	..
	Total	401	391	10	...	...	..
koliensis	Guadalcanal	205	...	40	117	48	..
	Hollandia	602	14	171	334	79	4
	Total	807	14	211	451	127	4
farauti	Espiritu Santo	398	203	194	1	...	..
	Guadalcanal	198	138	56	4	...	..
	Hollandia	129	14	65	43	7	..
	Morotai	42	7	13	18	4	..
	Total	767	362	328	66	11	..

TABLE 9.—*Larva: prothoracic submedian hairs*

Species	Locality	Hair 1—shape			Tubercles		
		Number examined	Per cent swollen	Per cent slender	Number examined	Per cent fused	Per cent separate
punctulatus	Guadalcanal	222	5.0	95.0	215	7.9	92.1
	Hollandia	135	1.5	98.5	140	2.1	97.9
	Total	357	3.6	96.4	355	5.6	94.4
koliensis	Guadalcanal	168	81.0	19.0	221	25.8	74.2
	Hollandia	565	91.7	8.3	587	93.9	6.1
	Total	733	89.2	10.8	808	75.2	24.8
farauti	Espiritu Santo	287	82.9	17.1	296	89.2	10.8
	Guadalcanal	181	87.3	12.7	192	93.2	6.8
	Hollandia	108	85.2	14.8	118	78.0	22.0
	Morotai	43	93.0	7.0	44	86.4	13.6
	Total	619	85.3	14.7	650	88.2	11.8

among wild and series larvae from each locality. When hair 1 of abdominal segment I has broad, notched leaflets, it is easily recognized as a true palmate tuft, although it is much smaller than the palmate hairs of the other segments. When it has hair-like branches, it does not resemble a palmate tuft at all; the branches are very slender, without a suggestion of broadening or flattening. Between these extremes are those branches which were designated as being slender; they were only slightly enlarged and flattened towards the base, and the hair did not resemble a true palmate tuft.

In *punctulatus,* the branches of hair 1 were usually short and hair-like (Fig. 8), occasionally slender, and rarely they were broad and notched. None of the *koliensis* hairs were of this nature; in these larvae the branches were sometimes slender but usually hair-like (Fig. 10). The broad, notched leaflets of the first abdominal palmate hairs of all *farauti* in Espiritu Santo and Guadalcanal makes it possible to identify the larvae of this species with complete certainty in these localities (Fig. 9). It is due to the fact that the first abdominal palmates of most of the *farauti* in Hollandia, and in some of those from Morotai, also have hair-like or slender branches; and to the shortening of the posterior clypeals in these areas, that it becomes impossible to differentiate between the larvae of *farauti* and *koliensis* in all parts of their ranges.

The branching of hairs 2 (antepalmate) and 6 (long lateral) of abdominal segments IV and V is summarized in Table 11. In *punctulatus,* 95.2 per cent of the

TABLE 10.—*Larva: hair 1, abdominal segment I*

Species	Locality	Source	Number examined	Structure of branches		
				No. broad, notched	No. slender	No. hair-like
punctulatus	Guadalcanal	Wild	28	2	..	26
		7 series	127	...	..	127
	Hollandia	Wild	116	1	36	79
		1 series	8	...	3	5
	Total	.....	279	3	39	237
koliensis	Guadalcanal	15 series	178	...	6	172
	Hollandia	Wild	91	...	2	89
		19 series	491	...	..	491
	Total	.....	760	...	8	752
farauti	Espiritu Santo	Wild	39	39	..	...
		19 series	183	183	..	...
	Guadalcanal	Wild	42	42	..	...
		8 series	74	74	..	...
	Hollandia	Wild	138	13	43	82
	Morotai	Wild	40	27	10	3
	Total	.....	516	378	53	85

antepalmates were three- to four-branched; in *koliensis* 88.0 per cent, and in *farauti* 81.7 per cent, were single or double. Hair 6 of *punctulatus* was three- to four-branched in 82.9 per cent of those examined; in *koliensis* 93.1 per cent, and in *farauti* 92.7 per cent were one- to two-branched. Thus, the antepalmate and long lateral hairs of abdominal segments IV and V are of some use in recognizing *punctulatus* larvae.

In summary, the larvae of *punctulatus* can be separated easily from those of *koliensis* and *farauti,* but it is often impossible to distinguish between the larvae of the latter species.

Differential characters of the pupae.—Although the pupal stage received no early attention in anopheline taxonomy, the comparatively recent works by Senevet (1930, 1931a and 1931b), Christophers (1933), Baisas (1935, 1936a and 1936b), and Crawford (1938) have shown the pupal stage to possess good differentiating characters.

TABLE 11.—*Larva: branching of hairs 2 and 6, abdominal segments IV and V*
Distribution according to number of branches

Species	Locality	Hair 2					Hair 6				
		Number examined	% 1	% 2	% 3	4 %	Number examined	% 1	% 2	% 3	% 4
punctulatus	Guadalcanal	432	...	1.9	96.5	1.6	445	..	13.7	84.3	2.0
	Hollandia	134	...	14.2	82.8	3.0	281	..	22.4	75.5	2.1
	Total	566	...	4.8	93.3	1.9	726	..	17.1	80.8	2.1
koliensis	Guadalcanal	330	0.9	72.1	27.0	..	355	0.6	89.0	10.1	0.3
	Hollandia	1053	6.3	86.4	7.3	..	810	2.0	92.6	5.3	0.1
	Total	1383	5.0	83.0	12.0	..	1165	1.6	91.5	6.8	0.1
farauti	Espiritu Santo	653	20.5	74.0	4.9	0.6	716	0.4	93.6	6.0	..
	Guadalcanal	343	25.1	65.0	9.9	..	340	8.8	90.0	1.2	..
	Hollandia	189	0.5	23.3	75.1	1.1	179	2.8	74.9	22.3	..
	Morotai	45	...	75.6	24.4	..	57	7.0	79.0	14.0	..
	Total	1230	18.0	63.7	17.8	0.5	1292	3.3	89.4	7.3	..

Several systems of pupal hair designation have been developed and used (see Baisas, 1938; and Edwards, 1941, for summaries of this work), but for at least two reasons were all considered to be unsatisfactory for use here: first, none of them include all of the known pupal hairs, and second, the systems all employ mixed types of name designations (words, numerals, and letters), which introduces unnecessary difficulties in the mechanical handling of a nomenclature. In an effort to overcome these undesirable conditions, an additional system was devised for this study.

The nomenclature introduced here (Figs. 11–14) consists of numerical designations, with no attempt being made to homologize the cephalothoracic hairs with those of the abdomen. In addition, no homologies are necessarily implied between the hairs of the abdominal segments, although every effort was made to recognize any possible affinities existing between them on various segments. In connection with this, those affinities established by the previous workers have been followed as much as possible.

Many difficulties are encountered in studying pupal chaetotaxy. The extremely small size of some of the pupal hairs is often a serious handicap in counting the number of branches. Often, more than one branch of a hair may be perfectly aligned on a vertical plane, or one or more branches may be broken off at the base. Some normally stout hairs occasionally have one or two branches extremely reduced in size. Fortunately, this is rare, because such minute branches are easily overlooked.

These are not to be confused with true feathered hairs which have never been observed on pupae of the *punctulatus* group. In studying the chaetotaxy, it is important to do both right and left sides of each specimen because occasionally a hair and even its ring may be completely missing from one side or the other, but never from both. A noticeable amount of natural variation in the position of hairs also occurs, the extent of which is rather difficult to determine, since the abdominal skin seldom lies perfectly flat in the mounting medium.

For a detailed discussion of the external morphology of the pupa, it is recommended that Crawford (1938) be consulted. Edwards (1941) discusses structures that have taxonomic value.

In order to determine possible diagnostic characters for the pupae, basic studies were made of the species treated here by selecting at random a series of at least 20 specimens of each (from Espiritu Santo and Guadalcanal) and tabulating the results obtained from examining and measuring all of the external morphological features. From a summary of these basic studies, it was possible to select all characters appearing to possess diagnostic value. These latter characters were then studied in

TABLE 12.—*Pupa: hair 8, cephalothorax*
Distribution by length in microns

Species	Number examined	51–70	71–80	81–90	91–100	101–110	111–120	121–130	131–140	141–190	% under 101 microns	% over 101 microns
koliensis	636	192	286	137	19	2	..	..	...	...	99.7	0.3
punctulatus	326	...	...	...	1	14	38	64	107	102	0.3	99.7
farauti	396	40	138	156	44	15	2	1	...	...	95.5	4.5

detail. The complete external morphology of a *farauti* pupa from Espiritu Santo is illustrated in Figs. 11–14.

An examination of the pigmentation of the pupal skin disclosed that a definite basic pattern exists among specimens from natural breeding places. Fig. 13 illustrates the pigmentation pattern of the cephalothorax of *farauti*. However, the use of the pattern as a diagnostic character is uncertain because there is so much variation in the amount of pigment deposited on it. In fact, except for the trumpets, no pigmentation pattern remained in pupae reared from eggs in the laboratory.

No character proved to be 100 per cent definitive between *punctulatus, koliensis* and *farauti*. The degree of intergradation between species varied considerably among the characters showing diagnostic value. However, for each species at least one character was found for which the percentage of intergrading specimens was low enough to allow correct identification of a high percentage of the material collected and studied. This identification could also be strengthened in most cases by utilization of subsidiary characters.

From the data collected it was found that *punctulatus* was the most distinct species, differing from the other two on the greater length of hair 8 of the cephalothorax (Table 12), on the greater number of branches of hair 10 of abdominal segment II (Table 14), and on the pigmentation of the trumpet. The trumpet of *punctulatus* was found to be pale or only slightly pigmented and offering little or no contrast to the cephalothorax in 96.0 per cent of the specimens examined, whereas in *koliensis* and *farauti* the trumpets were pigmented in contrast to the remainder

TABLE 13.—*Pupa: hair 5, abdominal segment II*
Distribution by length in microns

Species	Number examined	71–110	111–120	121–130	131–140	141–150	151–160	161–170	171–190	191–250	% under 161 microns	% over 161 microns
koliensis	242	36	44	51	76	31	4	..	...	..	100.0	...
punctulatus	232	..	1	..	2	4	10	25	115	75	7.4	92.6
farauti	288	22	51	74	66	41	16	12	5	1	93.8	6.2

of the cephalothorax in 99.0 per cent of the cases. The number of branches of hair 2 of abdominal segment III (Table 15) offers a supplementary character for *punctulatus* in that it was three- to five-branched in 32.6 per cent of the specimens examined, while in *koliensis* and *farauti,* less than one per cent were three-branched and none four- or five-branched. The greater length of hair 5 on segment II (Table 13), and of hair 1 of segments VI and VII (Tables 17 and 18) will separate *punctulatus* from *koliensis,* but not always from *farauti.* The shape of hair 1 of segments VI and VII of *punctulatus* (Fig. 19a) is generally quite distinct from

TABLE 14.—*Pupa: hair 10, abdominal segment II*
Number of branches

Species	Number examined	Range	Average	Per cent under 12 branches	Per cent 12 or more branches
koliensis	775	2–11	5.9	100.0	...
punctulatus	326	7–38	18.5	5.9	94.1
farauti	431	3–16	7.3	96.7	3.3

that of the other two species, being slenderer and longer. Hair 10 of abdominal segment VII and hair 12 of the paddles are longer in *punctulatus,* but the range of variation is sufficient to preclude their use for diagnosis.

Only one good character was found for the separation of *koliensis* and *farauti,* this being a comparison of the lengths of the lateral spines (hair 1) of abdominal segments IV and V. In *farauti,* the spine of IV was less than one-half the length of that of V in 89.4 per cent of the cases (if the Morotai specimens were excluded, this percentage would be 96.9), whereas in *koliensis,* the lateral spine of segment

TABLE 15.—*Pupa: hair 2, abdominal segment III*

Species	Locality	Source	Number examined	Number of branches				
				1	2	3	4	5
punctulatus	Guadalcanal	Wild	38	11	14	7	6	..
		8 series	134	21	53	42	16	2
	Hollandia	Wild	164	57	75	24	7	1
		1 series	7	..	..	6	1	..
	Total	.....	343	89	142	79	30	3
koliensis	Guadalcanal	23 series	185	178	7	..	..	..
	Hollandia	Wild	122	117	3	2	..	..
		19 series	116	109	7	..	..	..
	Total	.....	423	404	17	2	..	..
farauti	Espiritu Santo	Wild	28	28	...	..	..	..
		24 series	92	92	...	..	..	..
	Guadalcanal	Wild	74	35	35	4	..	..
		15 series	51	51	...	..	..	..
	Hollandia	Wild	153	150	2	1	..	..
	Morotai	Wild	57	57	...	..	..	..
	Total	.....	455	413	37	5	..	..

TABLE 16.—*Pupa: hair 1, abdominal segment IV*
Length in microns

Species	Number examined	Range	Average	Per cent under 32 microns	Per cent over 32 microns
punctulatus	357	10–62	20.5	97.5	2.5
koliensis	811	15–77	44.1	7.4	92.6
farauti	452	17–58	28.9	84.5	15.5

IV was one-half or more the length of the spine of V in 97.7 per cent of the specimens examined. The lateral spine of segment IV of *koliensis* (Table 16) averages considerably more in length than the same spine of *farauti,* but the range of variation is sufficient to make its use for identification difficult unless its length is compared with that of the spine of V. The shape of the lateral spines of segments VI and VII of *koliensis* (Fig. 19b) is generally distinct from the same ones of *farauti* (Fig. 19c). Fig. 16 illustrates a type of terminal indentation of the genitalia which occurs in a

TABLE 17.—*Pupa: hair 1, abdominal segment VI*
Length in microns

Species	Locality	Number examined	Range	Ave.	Per cent under 90 microns	Per cent over 90 microns
punctulatus	Guadalcanal	177	91–194	144	...	100.0
	Hollandia	172	91–184	137	...	100.0
	Total	349	.....	...	...	....
koliensis	Guadalcanal	190	51–119	75	97.2	2.8
	Hollandia	624	45– 92	73	95.2	4.8
	Total	814	.....	...	...	....
farauti	Espiritu Santo	125	78–140	112	9.6	90.4
	Guadalcanal	99	71–180	116	5.2	94.8
	Hollandia	155	57–138	91	41.3	58.7
	Morotai	57	61–153	88	57.9	42.1
	Total	436	.....	...	...	....

high percentage of the males of *koliensis.* Conditions approaching this, but seldom to be confused with it, may occasionally be observed in the males of *punctulatus* and *farauti,* but otherwise the genitalia of these last two are as illustrated in Fig. 14.

A possible effect of the environment, or perhaps the occurrence of a morphological and biological strain, is to be seen in the branching of hair 2 of abdominal segment III in *farauti* (Table 15). Wild reared material from Guadalcanal had this hair two- to three-branched in 52.7 per cent of the specimens studied, whereas it was single in all other *farauti* pupae examined, except for two specimens from Hollandia.

TABLE 18.—*Pupa: hair 1, abdominal segment VII*
Length in microns

Species	Locality	Number examined	Range	Ave.	Per cent under 102 microns	Per cent over 102 microns
punctulatus	Guadalcanal	177	98–204	153	0.8	99.2
	Hollandia	169	107–199	153	...	100.0
	Total	346	.....	...	...	....
koliensis	Guadalcanal	192	61–113	85	98.9	1.1
	Hollandia	612	51–126	83	95.5	4.5
	Total	804	.....	...	...	....
farauti	Espiritu Santo	127	78–160	109	30.7	69.3
	Guadalcanal	100	85–170	122	14.0	86.0
	Hollandia	147	74–169	104	49.7	50.3
	Morotai	56	61–153	99	63.9	36.1
	Total	430	.....	...	...	....

The constancy of characters among progeny from isolated females.—In Table 1, of the 269 *punctulatus* examined, 149 were from nine Guadalcanal and one Hollandia series. There was no variation in the proboscis markings, and all progeny were identical with the adults reared from wild-caught larvae.

There was practically no variation in the proboscis of *farauti*. Unfortunately, no series were obtained from Hollandia or Morotai; however, 707 of the specimens from Espiritu Santo and Guadalcanal belonged to 48 series, and of these, a single female from Espiritu Santo showed a few pale scales laterally on the proboscis towards the apex.

As shown above, the markings of the proboscis of *koliensis* are extremely variable. All of the 291 specimens from Guadalcanal belonged to 28 series, and of them, nine were identical to *farauti* in having a completely dark proboscis, while in seven the white markings were so extensive that they could not be separated from *punctulatus*. The same degree of variation was noted in *koliensis* from Hollandia. Of 293 specimens belonging to 19 series, three had a completely dark proboscis, while two had probosces similar to that of *punctulatus*.

The abnormal *koliensis* series described previously was the only one in which the progeny did not conform to the markings of the parent female. The other specimens that exhibited the extremes of proboscis markings appeared singly, or occasionally in pairs, in series of which the other members all had type C probosces. This haphazard occurrence suggests that such individuals are simply extremes of a variable mosquito, which happen to be similar to *punctulatus* on one hand and to *farauti* on the other.

In addition to the faithfulness of the progeny to the parent in proboscis markings, there was a tendency for certain characteristics to appear in larger numbers in some of the series. Thus, a female with an exceptionally dark type B palp might give rise to a series in which most of the individuals also had dark palpi.

In as much as the larval and pupal characteristics of the parent females were not known, it was not possible to compare the degree of variation between parent and offspring in these stages. However, just as the parent females with few exceptions did not have daughters that could be identified as belonging to another species, the female of a given species seldom produced a larva or pupa which could be mistaken for one of another species. Thus, *punctulatus* females always produced *punctulatus* larvae and pupae. In Guadalcanal, where *koliensis* and *farauti* larvae and pupae are distinct from one another, *farauti* females never produced *koliensis* larvae or pupae, and excepting the abnormal series, *koliensis* females never produced *farauti* larvae or pupae. In the abnormal *koliensis* series described above, the three larvae had first abdominal palmate hairs with slightly broadened leaflets. In Hollandia, *koliensis* females always gave rise to typical *koliensis* larvae and pupae, although these larvae could not be distinguished from most of those of *farauti* in that area.

Comparison of the species from different localities.—An examination of all the tables shows that there is no character in adults, larvae and pupae, by which *punctulatus* of Guadalcanal can be separated from *punctulatus* of Hollandia. A larger number of type C palpi was seen in the latter.

There is no character by which the *koliensis* of Guadalcanal can be distinguished from the *koliensis* of Hollandia. A difference was noted in the fusion of the prothoracic submedian tubercles of the larvae; in Guadalcanal 25.8 per cent were fused

while in Hollandia 93.9 per cent were fused. The form of the lateral spines of abdominal segments VI and VII of the pupa was not observed to intergrade with the *farauti* type in Guadalcanal, but this happened rather frequently in Hollandia material.

No important difference could be noted in the *farauti* adults from the four localities. A few of the specimens from Hollandia had type C palpi. In Espiritu Santo and Guadalcanal, 12.5 and 6.9 per cent respectively of the wings had one median spot, whereas in Hollandia 41.5 per cent of the wings had one median spot. The larvae from Espiritu Santo and Guadalcanal were identical, but they differed from those of Hollandia and Morotai in several respects. In the former areas, all but a few of the posterior clypeals extended to, or beyond, the anterior tubercles, while in Hollandia, only 19.8 per cent did so. The larvae from Morotai were similar to those of Hollandia in that only 42.2 per cent of the posterior clypeals reached the anterior tubercles. The *farauti* of Espiritu Santo and Guadalcanal were similar to *punctulatus* in having mostly one- to three-branched outer occipitals, but in Hollandia and Morotai most of these hairs were more like those of *koliensis* in being four- to six-branched. The most important difference between the *farauti* larvae of Espiritu Santo and Guadalcanal and of Hollandia and Morotai was in the development of the first abdominal palmate hair. Table 10 shows that in the first two localities, 100.0 per cent of these hairs had broad, notched leaflets, thus constituting an excellent character for separating *farauti* larvae from those of *koliensis* and *punctulatus*. In Hollandia, however, only 9.4 per cent of the first abdominal palmate hairs had broad, notched leaflets; in most of the larvae these branches were slender or hair-like, and indistinguishable from those of *koliensis*. In the Morotai *farauti,* most of the hairs had broad leaflets, but a number had slender or hair-like branches. Small differences were seen in the branching of the antepalmate (hair 2) and long lateral (hair 6) of abdominal segments IV and V (Table 11). Pupae of *farauti* from Hollandia and Morotai showed considerable variation from those of Guadalcanal and Espiritu Santo in several characters. Hair 1 of abdominal segments VI and VII of the pupa intergraded very considerably, both in length (Tables 17 and 18) and form, with those of *punctulatus*. Also, in comparing the lengths of the lateral spines of segments IV and V in *farauti,* it was found that the spine of IV was less than one-half the length of that of V in 96.9 per cent of the cases examined from Hollandia, Guadalcanal and Espiritu Santo but only in 71.9 per cent of those from Morotai.

Cross-mating experiments.—The abnormal *koliensis* series described above, in which most of the progeny had a dark proboscis, raises the question as to whether such progeny might be the result of hybridization. Lee and Woodhill (1944) believe that all of the intermediates might be hybrids of *punctulatus* and *farauti.*

Attempts were made to cross-breed *farauti* with *punctulatus* of Guadalcanal. Screened cages were placed out of doors under the shade of trees, and were covered with wet burlap in order to maintain a high humidity. Adults of *punctulatus* were taken from a laboratory colony maintained by Capt. John N. Belkin, AUS, while those of *farauti* were reared from larvae collected in a locality in Guadalcanal where this mosquito was breeding in pure culture. Upon emergence the males and females of *punctulatus* were placed respectively with the females and males of *farauti,* until there were several hundred adults in each cage. Under conditions similar to those

of this experiment, *punctulatus* colonized readily. A serious difficulty encountered was the refusal of the *farauti* females to feed; hence no progeny could be expected from any cross-mating of *punctulatus* males with *farauti* females. The *punctulatus* females attacked the human blood donors readily, but no hybrids were obtained from them. As soon as *punctulatus* males were placed with *punctulatus* females in these cages, fertile eggs were deposited. The strain of *farauti* evidently was one which did not adapt itself to laboratory conditions. This experiment was of too preliminary a nature to permit any conclusions, but it does appear that cross-breeding is not a phenomenon which occurs readily.

A NEW SPECIES OF THE PUNCTULATUS COMPLEX

On 22 March 1945, R. J. Clow, PhM2c, USNR, collected a larva from a road rut near Humboldt Bay, Hollandia, from which he bred out a female that proved to be unlike any other known *Neomyzomyia*. Although this single specimen is the only one available, it is felt that it would be an error not to include a description of the mosquito, as it obviously is a member of the *punctulatus* complex. It is being named in honor of the collector.

Anopheles (*Myzomyia*) *clowi*, new species

Adult Female.—A medium-sized anopheline with speckled and banded legs; length of wing, 3.5 mm.

Head: Vertex with a narrow, bare, longitudinal central stripe, on either side of which is a patch of upright black forked scales posteriorly and a patch of upright white forked scales anteriorly. Erect black fork scales laterally and centrally on occiput. Frontal tuft composed of long white hairs. Small appressed white scales along eye margin above. Antennae with pale brown torus and flagellar segments; small white scales on first flagellar segment, whorl hairs pale. Clypeus bare, brown. Palpi (Fig. 36) equal to proboscis in length; segment II dark with a narrow white apical ring and a few pale scales centrally on dorsal surface; segment III with some paler brown scales on inner surface towards the base, a small subapical pale spot, and a narrow apical band of yellowish scales; segment IV with the basal one-third black, apical two-thirds yellowish; segment V with a narrow black basal ring, the remainder yellowish. Proboscis (Fig. 36) with labium dark except for a small ventral patch of yellowish scales near apex; labella yellow.

Thorax: Median area of scutum grey-pollinose; prescutellar area bare, dark brown; lateral areas darker brown; brown eyespot at scutal angle. Scutum clothed with broad white scales and pale bristles; a small patch of dark scales on antero-lateral angle. Prothoracic lobes with a patch of dark scales above; posterior pronotum bare. Propleuron with a single hair. Spiracular bristles absent. Postspiracular area bare; sternopleuron with a row of small scales along posterior border. Mesepimeron with a small patch of pale scales at upper margin. Pleurae with a longitudinal dark brown stripe on upper border from posterior pronotum to mesepimeron; a brown spot on lower mesepimeron; meron and lower portion of sternopleuron dark brown. Scutellum largely denuded, apparently with some narrow pale scales in addition to the hairs.

Wings (Fig. 35): Costa with three pale humeral spots; sectoral pale spot large but accessory sectoral spot not present on costa or subcosta; subcostal spot large, slightly smaller than dark area between it and the sectoral spot; preapical spot equal to the dark area between it and the subcostal spot; apical spot extending from above the termination of the first vein to the apex of vein 2.1. Vein 1 with six small dark spots between the base and the accessory sectoral spot, a broader dark area below the median large dark area of the costa, then five small dark spots to the subapical dark area, which is similar to that on the costa. Stem of vein 2 pale, with a small dark spot near base and another beyond the middle; a small dark spot at the fork in one wing, fork pale in the other; vein 2.1 pale, with two small dark spots and a larger subapical one corresponding to the subapical dark area of the costa and vein 1, the apex pale; vein 2.2 pale with four small dark spots and a fifth at the apex. Vein 3 pale, with seven small dark spots; apex pale. Vein 4 with only a few narrow pale and dark scales basad of the anterior cross vein, a few pale scales at the cross vein, the rest of the stem dark to the fork; vein 4.1 with a long dark area beyond pale spot at the fork, followed by a small white spot, a small dark spot, and a longer pale area to tip of vein; vein 4.2 with a small dark spot beyond the pale spot at the

fork, then alternating two white and two dark spots, and white at tip. Vein 5 pale; stem with six small dark spots; the fork pale; vein 5.1 with five small dark spots; vein 5.2 with four small dark spots. Vein 6 with six small dark spots. Fringe spots opposite veins 1, 2.1, 2.2, 3, 4.1, 4.2, 5.1, 5.2, and 6; a spot also between 5.1 and 5.2, 5.2 and 6; two small and one large pale spots between tip of vein 6 and base of wing. Halter with white-scaled stem, apex of knob dark-scaled.

Legs: All coxae with a few pale scales. Foreleg (Fig. 37) : femur swollen on basal half, the dorsal and ventral surface with about 10 irregular white patches, which become more distinct and band-like distally, a narrow pale ring at tip; tibia with about 10 irregular small pale spots on anterior and posterior surfaces, becoming larger distally; first tarsal segment mottled with large dark and yellow spots and with a yellow apical ring; second, third, fourth, and fifth tarsal segments entirely yellow except for a narrow black ring at the base of each. Midleg: femur with about 10 small pale spots on dorsal surface which are larger and confluent basally, the ventral surface with an irregular but almost continuous pale stripe; tibia broadly mottled dark and yellow; first tarsal segment mottled with dark and yellow spots, and with a narrow apical yellow ring; second, third, tourth, and fifth tarsal segments entirely yellow except for a narrow basal ring on each. Hind legs missing.

Abdomen: Tergites I and II without scales; III with a few narrow pale scales in center near apical border; IV with similar scales centrally near apical border; V, VI, VII, and VIII with progressively heavy clothing of narrow white scales. Sternite I without scales; II to VII with scattered pale and dark scales, VII also with an apico-ventral tuft of black scales; VIII rather heavily pale-scaled. All segments with long pale hairs. Cerci clothed with light and dark scales.

LARVA.—*Head:* Inner anterior clypeal hairs widely spaced, with a few small lateral spicules; outer anterior clypeals simple, one-fourth the length of the inner; posterior clypeals single, extending beyond tubercles of anterior clypeals. Antennal hair minute; terminal hair of antenna three- to four-branched; inner surface of antenna with short, stout spines becoming progressively larger distally. Inner occipital hairs long, single; outer occipitals missing.

Thorax: Prothoracic hair 1 (Fig. 39) with a long, slender stem, with 14–15 lateral branches; hair 2 missing; hair 3 short, single; tubercles of hairs 1 and 2 relatively large but separated from one another. Prothoracic pleural group with three long, single hairs; hair 11 single, about a third the length of the long hairs. Mesothoracic pleural group with two long and one shorter single hairs; hair 11 minute. Metathoracic pleural group with two long and one short single hairs, hair 11 minute. Thoracic palmate hair (Fig. 38) with elongate pedicel and five or six very long, slender, attenuated leaflets.

Abdomen: Hair 1 of segment I with seven or eight long, hair-like branches; hair 1 of segment II with broad, notched leaflets, somewhat smaller than the fully developed palmates of segments III to VII. Filament of leaflet short, one-half the length of the blade (Fig. 40). Hair 2 of segments IV and V single; hair 6 of these segments triple. Tergal plates well pigmented, smaller on anterior segments; those on segments V–VII about three-fourths as wide as the distance between the tubercles of the palmate hairs; tergal plate on VIII large; median accessory plaques on segments II–VII. Pecten spines mostly subequal in length.

PUPA.—*Cephalothorax:* Trumpet palely pigmented (slightly more so than in *punctulatus*) over entire length, offering only slight contrast to remainder of cephalothorax; ratio of depth of basal notch to length of trumpet approximately 1 : 1.4. Ratio of width to length of head shield 1 : 0.88. External margin of palpal case angulate. Metanotum with a distinct median bar, bounded laterally by a crease which is nearly complete posteriorly.

Abdomen: Hair 5 of segment II stout, dark; hair 10 of II (Fig. 41) distinctive in possessing a dark, stout, basal stalk which is approximately one-third total length of hair; hair 5 of segment IV external to hair 8; hair 2 of segments VI and VII anterior to the posterior margin of the segment. Lateral spine (hair 1) of segment IV (Fig. 42) approximately twice the length of that on III; lateral spines of V, VI and VII several times the length of that on III, and over twice the length of that on IV; lateral spines of IV–VII darkly pigmented, stout, smoothly and acutely tapered, those on VI and VII (Figs. 43 and 44) bearing a distinct, constricted rugose area near base.

Paddle: Midrib with external margin distinct to posterior two-thirds, with scanty granulations. Buttress well developed, external marign of paddle distinct to posterior one-half and possessing minute, clear spinules that increase in size posteriorly to beginning of fringe. Fringe of fine pale hairs extending from center of external margin to the beginning of anterior curve internal to hair 12, longest just external to this hair and much reduced right at hair 12. Hair 12 straight, rather stout, darkly pigmented.

Genitalia: No variation from female genitalia typical of the other members of the *punctulatus* complex.

PERTINENT DETAILS OF CHAETOTAXY OF *Anopheles clowi*

Body area	Hair number	Length in microns	No. of branches	Body area	Hair number	Length in microns	No. of branches
C. thorax	8	153, 167	...	Abd. V	1	82, 88	...
" "	10	177, 180	...	" "	2	.....	1, 1
Abd. I	6	.....	2, 4	" "	8	.....	2, 4
" "	9	.....	1, 1	" "	10	.....	2, 2
Abd. II	5	204, 211	1, 1	Abd. VI	1	75, 82	...
" "	10	.....	17, 18	" "	6	.....	4
Abd. III	1	14, 17	...	" "	8	.....	3, 3
" "	5	.....	2, 2	" "	10	.....	1, 3
" "	8	.....	11, 11	Abd. VII	1	78, 88	...
" "	10	.....	9, 9	" "	6	.....	4, 4
Abd. IV	1	31, 37	...	" "	8	.....	3, 3
" "	2	.....	1, 1	" "	10	306, 382	1, 2
" "	8	.....	4, 4	Abd. VIII	8	.....	3, 3
" "	10	.....	3, 4	Paddle	12	122	...

TYPE.—Holotype: ♀, with larval and pupal skin, Hollandia, Humboldt Bay, Dutch New Guinea, 22 March 1945 (R. J. Clow). *Holotype* to be deposited in the U. S. National Museum, Washington, D. C.

Taxonomic discussion.—The spotting of the femora and tibiae, presence of a propleural hair, lack of spiracular bristles, presence of a scale tuft on the prothoracic lobes, and simple pleural hairs of the larva make *A. clowi* a member of the *Neomyzomyia* Group. *A. clowi* can be distinguished from *punctulatus, koliensis, farauti,* and *annulipes* by tarsal segments II to V of the fore and mid legs, which are entirely yellow except for a small basal black ring on each, while in the other species, although an occasional segment may be completely white, some and usually all of the fore and mid-tarsi are dark, ringed, or spotted black and white. Another difference between *clowi* and the other species is the extra fringe spots of the wing. The larva of *clowi* is very similar to that of *punctulatus,* but prothoracic hair 1 is somewhat longer in *clowi,* the thoracic palmate leaflets are longer and more attenuated, and the second abdominal palmates are better developed, being only a little smaller than the palmates of segment III. The larva of *annulipes* has branched outer anterior and posterior clypeal hairs, which separate it from that of *clowi.* The pupa of *clowi* differs from those of *farauti* and *koliensis* in the greater number of branches of hair 10 of abdominal segment II and in the greater length of hair 8 of the cephalothorax, and from *punctulatus* by the more distal branching of hair 10 of abdominal segment II, and the rugose constriction at the base of the lateral spine of abdominal segment VII.

Other than these five species, the only *Neomyzomyias* which have a complete clothing of scales on the scutum and a dark-scaled halter knob are *meraukensis, kochi, amictus amictus,* and *amictus hilli.* All of these mosquitoes have dense scaling on the abdominal tergites. Other characters which serve to distinguish between them and *clowi* are the heavily branched outer clypeals of *meraukensis;* the ventral abdominal scale tufts, palpal markings, wing pattern, and small outer clypeals of *kochi;* the 10–14 propleural hairs of the adults, and branched pleural hairs of the larvae of *amictus amictus* and *amictus hilli.*

A. novaguinensis also has a dark-scaled halter, but the scutal scaling is restricted to the central area only, and one of the long hairs in each of the pleural groups of the larva is branched.

DISCUSSION

There is no reason to assume that there is any difference between the *farauti* of the New Hebrides, Guadalcanal, Hollandia, and Morotai. Lee and Woodhill (1944) believe that the Buxton and Hopkins (1927) description of the larva from the New Hebrides indicates that Laveran's mosquito might represent a different variety, but of the several hundred larvae that were examined from the eastern part of the range, not one was seen that had the very short outer clypeal hairs illustrated by Buxton and Hopkins. It is true that no material was available from Mallekula, where these authors collected the larva they illustrated. However, no such hairs were seen in larvae from Efate, the type locality of Laveran's *farauti*. The only differences observed between the *farauti* from the eastern and western parts of the range are believed not to be constant enough to indicate the existence of two varieties of the dark-proboscis mosquito. The broad leaflets could hardly define the New Hebrides larva as a separate variety from *moluccensis,* for Swellengrebel and Rodenwaldt (1932) illustrate a *moluccensis* larva with extremely well developed palmates on the first abdominal segment, and the authors' own collections in the Netherlands Indies show that such larvae are present in the western part of the range. The name *moluccensis,* therefore, becomes a synonym of *farauti* Laveran.

The exact taxonomic positions of *farauti, punctulatus,* and *koliensis* cannot be ascertained with certainty without additional cross-breeding experiments. The possible interpretations of their status are as follows:

First, that they are all biotypes of a polymorphic species, and interbreed freely, producing offspring which exhibit any of the characters described above. One could easily visualize an area somewhere in coastal New Guinea, where the species probably originated, in which all possible genetic combinations exist. As the species migrated from this center of origin, opportunities occurred for isolation of certain strains. At higher elevations, the white-proboscis *punctulatus* type became predominant. In other areas, particularly in the islands at the eastern limit of the range, the dark-proboscis strain established itself. When observed in their isolated habitats, these two strains would appear distinct enough from one another in larval, pupal, and adult stages to merit specific rank. *A. koliensis* might represent a third strain, which, with one possible exception, had not had the opportunity for isolation, and so has not developed characters in any region which would lead one to consider it a true species. The exception may have occurred in Guadalcanal, where Owen (1945) discovered this mosquito in the Koli Area early in 1944. A few months earlier, one of us (KLK) had made surveys in this area without finding the mosquito, which would suggest that its introduction into Guadalcanal was a recent one, and was accomplished by one or a few individuals, perhaps by means of military aircraft. There are airfields in the region where *koliensis* was discovered. The strain thus isolated was distinct in larval, pupal, and adult stages from the isolated strains of *farauti* and *punctulatus* on the same island, and so were considered by Owen (1945), and by Belkin, Knight and Rozeboom (1945) to represent a full species.

In retracing the paths of migration back to the center of origin, the distinctive characters would begin to blend. With increasing opportunity for genetic combinations, parent females would produce offspring with any of the morphological characteristics at first thought to be of specific quality. The hesitancy of the authors to accept this theory is based upon their failure to collect in an area where reared

progeny showed a considerable degree of divergence from the parent female. Such an area may well exist, but if so, even Hollandia in New Guinea must be far enough removed, ecologically if not geographically, to inhibit indiscriminate intermingling of the three forms.

A second interpretation, as advanced by Lee and Woodhill (1944), is that *punctulatus* and *farauti* are either subspecies or species, but that the intermediate *koliensis* is a hybrid of the first two. The authors' limited experiments did not suggest that cross-mating takes place readily, which it should do if the dense *koliensis* populations in some areas are to be explained by hybridization. As stated above, extensive cross-breeding experiments are necessary before this theory can be rejected or accepted. Should cross-breeding produce hybrids with intermediate characters, progeny must be obtained from these hybrids to determine whether such characters are perpetuated in the offspring. This is what occurred in the long series of *koliensis* progeny reared by the authors, if it is assumed that the pregnant, parent females captured in houses were hybrids. Hybridization would thus produce a strain genetically distinct from both parent strains. Dobzhansky (1941) points out that ". . . introgressive hybridization seems to be much commoner in the plant than in the animal kingdom."

Finally, the three forms could be genetically distinct, and hence merit specific or subspecific rank. Failing to discover an area where blending of the strains was apparent, and without success in attempts to cross-breed *farauti* and *punctulatus,* it is felt that the data presented in this paper support the last interpretation.

The purpose of rearing progeny from isolated females was to determine the validity of the characters used for separation of the species by observing the variability of such characters within each series. If variations were to occur, they would indicate that the "species" or "subspecies" were merely opposite extremes of a single polymorphic species. Thus, if a dark-proboscis *farauti* female were to produce daughters with the small ventral pale patch on the proboscis, it could be concluded that the Dutch workers were correct in assuming that both of the types of adults belonged to their variety *moluccensis.* On the other hand, if the characteristics of the progeny remain faithful to those of the parent female, with dark-proboscis adults producing dark-proboscis progeny, and adults with the ventral white patch on the proboscis producing similar progeny, it would seem that the two forms are systematically distinct. With few exceptions, the results of the progeny rearings support the theory that the three forms are genetically distinct. *A. punctulatus* progeny showed no *koliensis* characters, and only once was a proboscis somewhat like that of *koliensis* seen in *farauti* progeny. Neither *punctulatus* nor *farauti* ever produced daughters that could be confused with one another. Since the proboscis markings of *koliensis* are so variable, it is not surprising that some specimens could attain both extremes of ornamentation. However, these extremes represented only a small percentage of the total. It has been pointed out that most of the dark-proboscis individuals of *koliensis* belonged to a single short series. The parent female had a rather large subapical ventral patch of white scales on the proboscis, but among nine female offspring, six had a *farauti*-like proboscis, and only three were of type C. Three larval skins of the series were available for study, and they too showed *farauti*-like characters in the shape of the leaflets of hair 1 of the first abdominal segment, in being somewhat swollen rather than hair-like. The pupal

skins associated with the three larval skins all possessed *koliensis* characters. If similar results had been obtained with the progeny of additional *koliensis* females, the evidence would strongly support the concept of the Dutch workers that their forms and *farauti* all represent a single entity. However, the fact remains that the dark proboscis appeared in significant numbers among the progeny of only one out of 47 *koliensis* females. The other six dark-proboscis *koliensis* occurred singly or in pairs among four series. It is not impossible that the abnormal series resulted from cross-mating of *farauti* and *koliensis*.

If it is conceded that the progeny rearings prove the existence of three genetically distinct systematic entities, the decision must be made whether to call them species or subspecies. In either case, the three forms should be given equal rank, for on adult characters, it would be difficult to designate *koliensis* as a variety of either of the other two, since 12 daughters in the reared series had type A and 9 had type B probosces. It is true that the pupae of *koliensis* are similar to, and the larvae are indistinguishable from those of *farauti,* in the western part of the range. This close affinity might be expressed by considering *koliensis* to be a variety of *farauti.* However, in conformity with the concept of subspecies or varieties being separated geographically or ecologically, the three forms are considered to be full species. They may be found in the same breeding waters; for example, in Hollandia, some pools were found at the margins of a creek, from which a total of 154 females were bred out with associated larval and pupal skins. The populations here were as follows: *farauti,* 33.8 per cent; *koliensis,* 31.1 per cent; and *punctulatus,* 35.1 per cent. If one were to rely only upon wild caught larvae, the inevitable conclusion would be that two species, *farauti* and *punctulatus,* were present in the pools, separable by larval characters, and that the *farauti* larvae gave rise to adults of which 52.0 per cent had completely dark and 48.0 per cent had slightly pale-scaled probosces. Such results are similar to those which led de Rook (1924) to conclude that *moluccensis* adults could possess either a completely black proboscis or one with a subapical, ventral pale area. In the reared progeny, however, one sees an entirely different picture, in that the proboscis markings are constant among offspring from a given female. Furthermore, pupal characters are constant among progeny.

A. annulipes was not included in this study, for there was no opportunity to collect within its range . Because it is an extremely variable species, similar observations on it should furnish interesting results.

Belkin, Knight, and Rozeboom (1945) point out that *A. punctulatus, farauti,* and *koliensis* form a complex of species which are separated from all other Australasian and Oriental *Neomyzomyias* by a combination of uniform scutal scaling, abdominal scaling restricted to terminal segments, broad apical pale bands on the last two palpal segments; larvae with unbranched pleural hairs, inner clypeals widely spaced and simple or slightly frayed, outer clypeals single, frayed, or occasionally branched but never minute, and posterior clypeals extending at most only slightly beyond the clypeus. To these species should be added *clowi,* for it meets all the above specifications except that it possesses sparse scaling on some of the proximal abdominal segments. The classification of the *punctulatus* complex is considered to be as follows:

A. punctulatus Dönitz, 1901.

A. farauti Laveran, 1902.

Syn.: *A. moluccensis* Swellengrebel and Swellengrebel de Graaf, 1920.

A. koliensis Owen, 1945.

Syn.: *A. moluccensis,* in part, of Edwards, 1921, de Rook, 1924, and of Swellengrebel and Rodenwaldt, 1932.

A. clowi, new species.

The results of this study show that Owen's *koliensis* from Guadalcanal is identical with the intermediate specimens identified by the Dutch workers as *moluccensis,* and which comprised 96 per cent of the material described by Swellengrebel and Rodenwaldt (1932). The reluctance to synonomize the old and familiar name of *moluccensis* might lead one to apply it to the intermediate form. But in its original sense it obviously is a synonym of *farauti,* and so for the present, the authors are employing Owen's name of *koliensis.*

KEYS TO SPECIES

Adult females

1. Labium completely dark-scaled, rarely with a few ventral pale scales on basal half ..... *A. farauti*

   Pale scales present on apical third of labium 2

2. A small pale area on ventral surface of labium near the tip, or a few subapical ventral pale scales 3

   Labium more extensively pale-scaled, with white markings on the dorsal as well as on the ventral surface 4

3. Tarsal segments II–V of fore and mid legs completely yellow except for a narrow black ring at the base of each; abdominal scaling present on tergites III–VIII and on sternites II–VIII; wing with fringe spots at tips of veins and also between veins 5.1 and 5.2, 5.2 and 6, and two short and one long fringe spots between 6 and base of wing ..... *A. clowi*

   Tarsal segments II–V of fore and mid legs never as above; instead, some of these segments entirely dark, others dark with a narrow basal or apical, or both basal and apical pale bands; abdominal scaling on terminal segments only; fringe spots at tips of veins only ..... *A. koliensis* (in part)

4. Dorsal pale markings on labium restricted to a narrow band extending upwards from the distal border of the small ventral pale area .. *A. koliensis* (in part)

   Dorsal pale markings of labium more extensive 5

5. Pale markings of labium irregular, the ventral marking more extensive than that on the dorsal surface, or the dorsal pale area more extensive than that on the ventral surface; an oblique line of demarcation between dark and light areas on lateral surface of the labium; wing usually with only one pale median spot; palpus seldom with three broad pale bands on last three segments, segment III often with one or both of the apical and subapical pale markings reduced to a few scales, or entirely absent; tarsal segments darker ..... *A. koliensis* (in part)

   Labium with the apical half or one-third evenly pale-scaled dorsally and ventrally, with or without a small dark ring before the labella; wing usually with both sectoral and accessory sectoral pale spots, which sometimes are fused into a single broad pale area; palpus often with three broad apical pale bands, pale markings on segment III rarely reduced; tarsi paler, some segments mottled or even completely white ..... *A. punctulatus*

Fourth-stage larvae

1. Hair 1 of abdominal segment I a true palmate tuft, with broad, flattened, notched leaflets ..... *A. farauti* (in part)
   This hair not resembling a palmate hair, but with slenderer or narrow, hair-like branches 2
2. Stem of prothoracic hair 1 slender; tubercles of prothoracic hairs 1 and 2 small, unpigmented, and well separated from one another; posterior clypeal hairs extending to or beyond the tubercles of the anterior clypeals; inner occipital hairs usually single; hairs 2 and 6 of abdominal segments IV and V usually three- to four-branched; spines of pecten usually subequal; outer anterior clypeal hairs single, often less than half the length of the inner clypeals ... 3
   Prothoracic hair 1 with stout, swollen stem; tubercles of prothoracic hairs 1 and 2 large and generally fused together; posterior clypeal hairs often not reaching tubercles of anterior clypeals; inner occipital hairs usually two- to five-branched; hairs 2 and 6 of abdominal segments IV and V usually one- to two-branched; spines of pecten with irregular arrangement of long and short teeth; outer anterior clypeal hairs often branched and frequently over half as long as the inner clypeals ..... *A. koliensis; A. farauti* (in part)
3. Second abdominal palmate hairs well developed, with broad leaflets, only a little smaller than the palmate hairs of segment III; prothoracic hair 1 quite long; leaflets of metathoracic palmate hair very long and tapering ..... *A. clowi*
   Second abdominal palmate hairs small, leaflets narrow, prothoracic hair 1 shorter; leaflets of metathoracic palmate hair shorter ..... *A. punctulatus*

Pupae

1. Hair 10 of abdominal segment II with 13 or more branches; hair 8 of cephalothorax over 105 microns in length 2
   Hair 10 of abdominal segment II with less than 13 branches; hair 8 of cephalothorax less than 105 microns in length 3
2. Hair 1 (lateral spine) of abdominal segment VII smooth, slender (Fig. 19a), averaging 153 microns in length; hair 10 of segment II branched from near base ..... *A. punctulatus*
   Hair 1 of abdominal segment VII stout, with a distinct rugose constriction near base (Fig. 44), averaging approximately 82 microns in length; hair 10 of segment II with main branching arising at basal one-third from a stout stalk (Fig. 41) ..... *A. clowi*
3. Hair 1 of abdominal segment IV less than one-half the length of that of segment V ..... *A. farauti*
   Hair 1 of abdominal segment IV one-half or more the length of that of segment V ..... *A. koliensis*

SUMMARY AND CONCLUSIONS

Detailed morphological studies were made of the larvae, pupae, and adult females of *A. punctulatus, koliensis,* and *farauti* from Espiritu Santo in the New Hebrides, Guadalcanal in the Solomon Islands, Hollandia in Dutch New Guinea, and Morotai in the Moluccas. The material consisted of associated larval, pupal, and adult specimens collected as larvae from natural breeding places, and of laboratory-reared progeny from individual females.

Principally on the basis of the coloration of the proboscis, *A. punctulatus, koliensis,* and *farauti* are considered to be systematically distinct. *A. punctulatus,* separable from the others in larval, pupal, and adult stages, is considered to be a full species. The constancy of the proboscis pattern among all but a few of the progeny of *farauti* and *koliensis* is interpreted to mean that these two are distinct from one another, and although they are much more closely related to one another than to *punctulatus,* because they can be found together in the same breeding waters, they are considered to be of specific rank.

The *farauti* of Espiritu Santo, Guadalcanal, Hollandia in Dutch New Guinea, and Morotai in the Moluccas, all represent a single species; hence *moluccensis* Swellengrebel and Swellengrebel de Graaf, 1920, becomes a synonym of *farauti* Laveran, 1902.

The *koliensis* of Guadalcanal and the "intermediate form" of New Guinea are the same, and if cross-breeding experiments show that this mosquito is not a hybrid, the name *koliensis* must be applied to it.

A. punctulatus of Guadalcanal is identical with the *punctulatus* of Hollandia.

A. clowi, a new member of the *punctulatus* complex, is described.

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Explanation of Plates

Plate I

Fig. 1. *A. farauti.* Dorsal aspect of larval head.
Fig. 2. " " Larval thorax.
Fig. 3. " " Abdominal segments I–III of larva.

Plate II

Fig. 4. *A. farauti.* Abdominal segments IV–X of larva.

Plate III

Fig. 5. *A. farauti.* Prothoracic submedian hairs of larva.
Fig. 6. *A. koliensis.* Prothoracic submedian hairs of larva.
Fig. 7. *A. punctulatus.* Prothoracic submedian hairs of larva.
Fig. 8. " " Hair 1 of abdominal segment I of larva.
Fig. 9. *A. farauti.* Hair 1 of abdominal segment I of larva.
Fig. 10. *A. koliensis.* Hair 1 of abdominal segment I of larva.

Plate IV

Fig. 11. *A. farauti.* Metanotum and abdominal segments I–IV of pupa.
Fig. 12. " " Abdominal segments V–VIII of pupa
Fig. 13. " " Cephalothorax of pupa.
Fig. 14. " " Anal segments of pupa. Male.
Fig. 15. *A. punctulatus.* Trumpet of pupa.

Plate V

Fig. 16. *A. koliensis.* Anal segments of pupa. Male.
Fig. 17. *A. farauti.* Anal segments of pupa. Female.
Fig. 18. *A. punctulatus.* Dorsal aspect of abdominal segment IV of pupa.
Fig. 19. Lateral spines (hair 1) of abdominal segment VII of pupa.
a. *A. punctulatus.*
b. *A. koliensis.*
c. *A. farauti.*

Plate VI

Fig. 20. *A. farauti.* Proboscis, type A.
Fig. 21. *A. punctulatus.* Proboscis, type B.
Fig. 22. " " Proboscis, type B.
Fig. 23. *A. koliensis.* Proboscis, type C.
Fig. 24. " " Proboscis, type C.
Fig. 25. " " Proboscis, type C.
Fig. 26. " " Proboscis, type C.
Fig. 27. " " Proboscis, type C.
Fig. 28. Palpus, type A.
Fig. 29. Palpus, type A.
Fig. 30. Palpus, type B.
Fig. 31. Palpus, type B.
Fig. 32. Palpus, type B.
Fig. 33. Palpus, type B.
Fig. 34. Palpus, type C.

Plate VII

Fig. 35. *A. clowi.* Wing.
Fig. 36. " " Proboscis and palpus.
Fig. 37. " " Tarsus of fore leg.
Fig. 38. " " Metathoracic palmate hair of larva.
Fig. 39. " " Prothoracic hair 1 of larva.
Fig. 40. " " Leaflet of abdominal palmate hair of larva.
Fig. 41. " " Hair 10 of abdominal segment II of pupa.
Fig. 42. " " Hair 1 of abdominal segment IV of pupa.
Fig. 43. " " Hair 1 of abdominal segment VI of pupa.
Fig. 44. " " Hair 1 of abdominal segment VII of pupa.

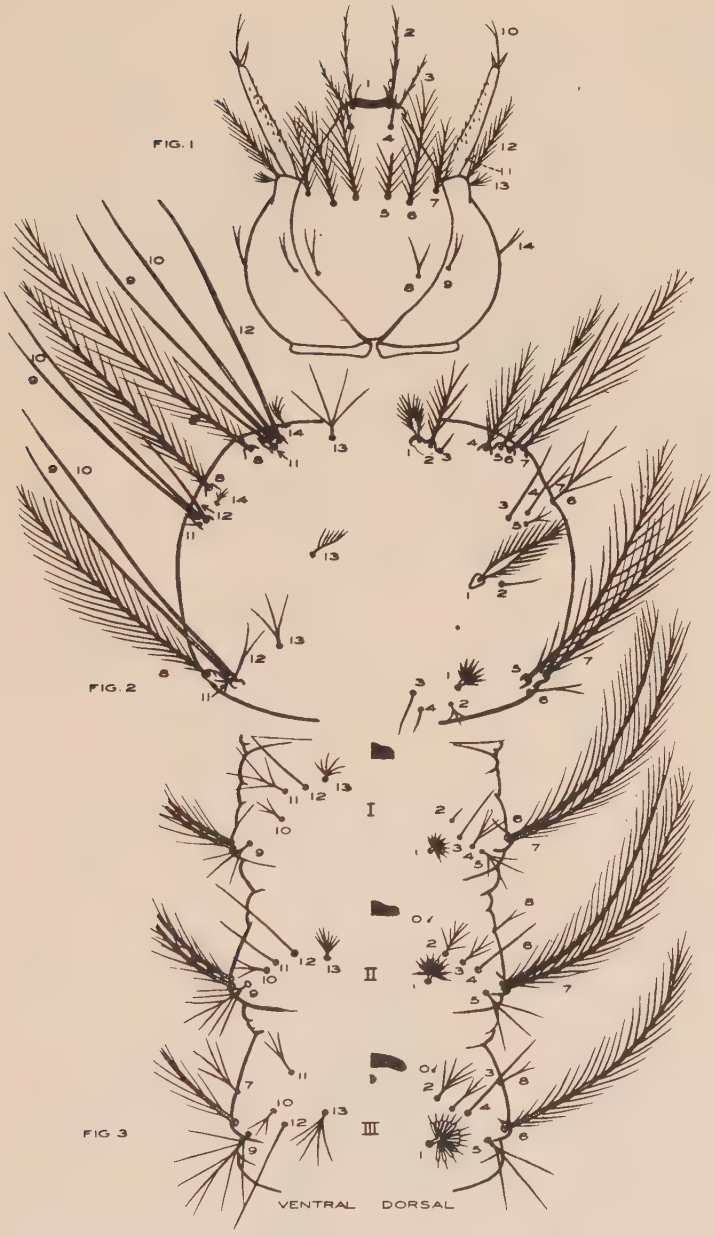

PLATE I

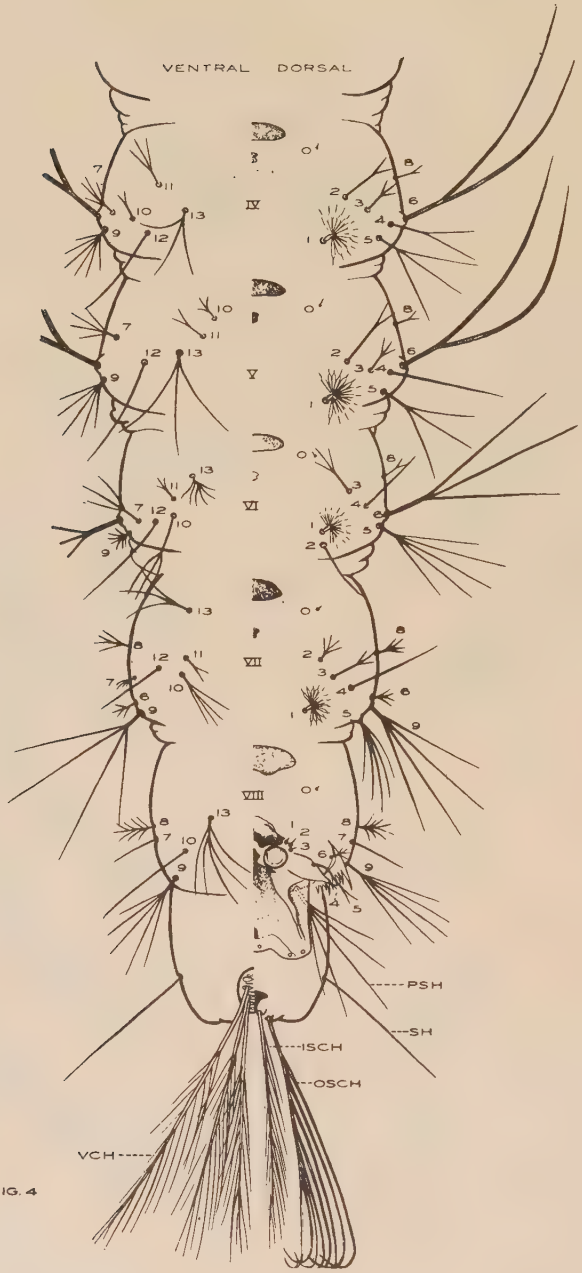

FIG. 4

PLATE II

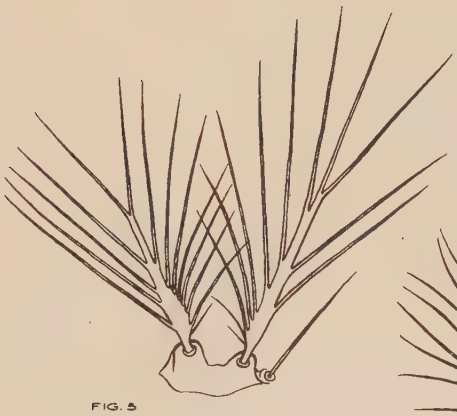

FIG. 5

FIG. 6

0.05 MM.

FIG. 7

0.05 MM.

FIG. 8

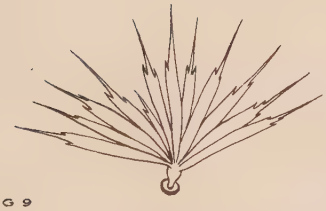

FIG 9

0.10 MM.

FIG 10

0.10 MM.

PLATE III

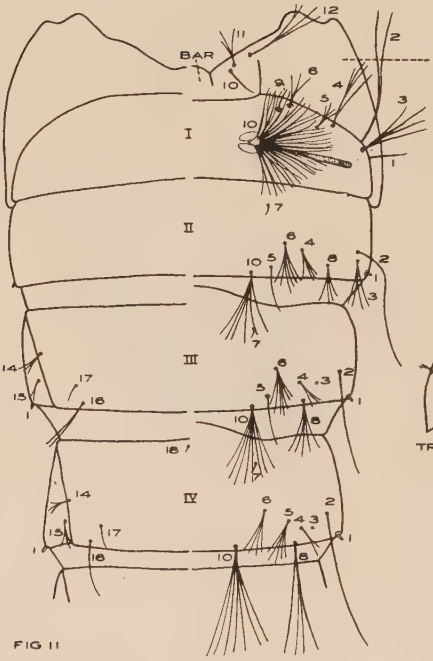

FIG 11

FIG 12

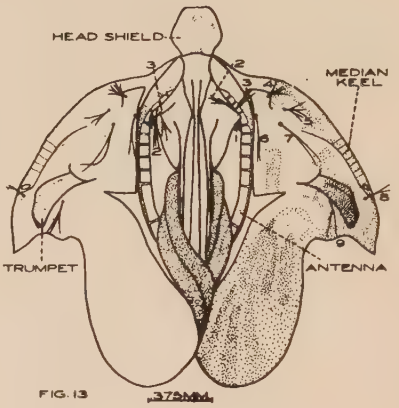

FIG. 13

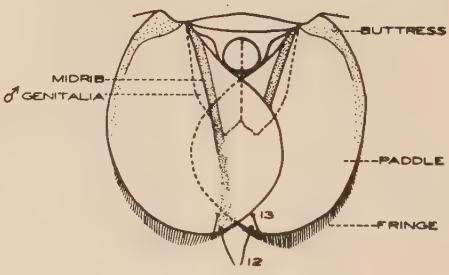

FIG. 14

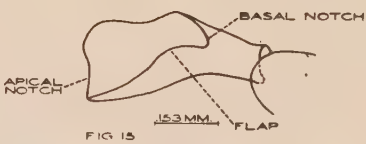

FIG 15

PLATE IV

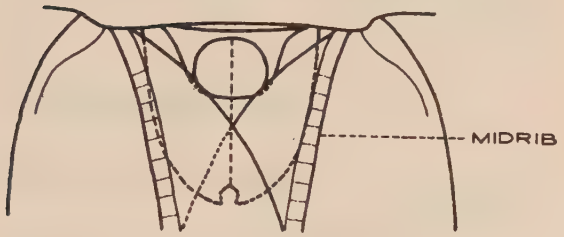

FIG. 16

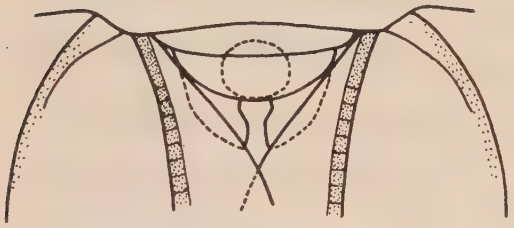

FIG. 17

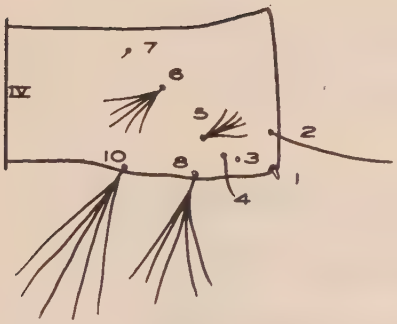

FIG. 18

FIG. 19

PLATE V

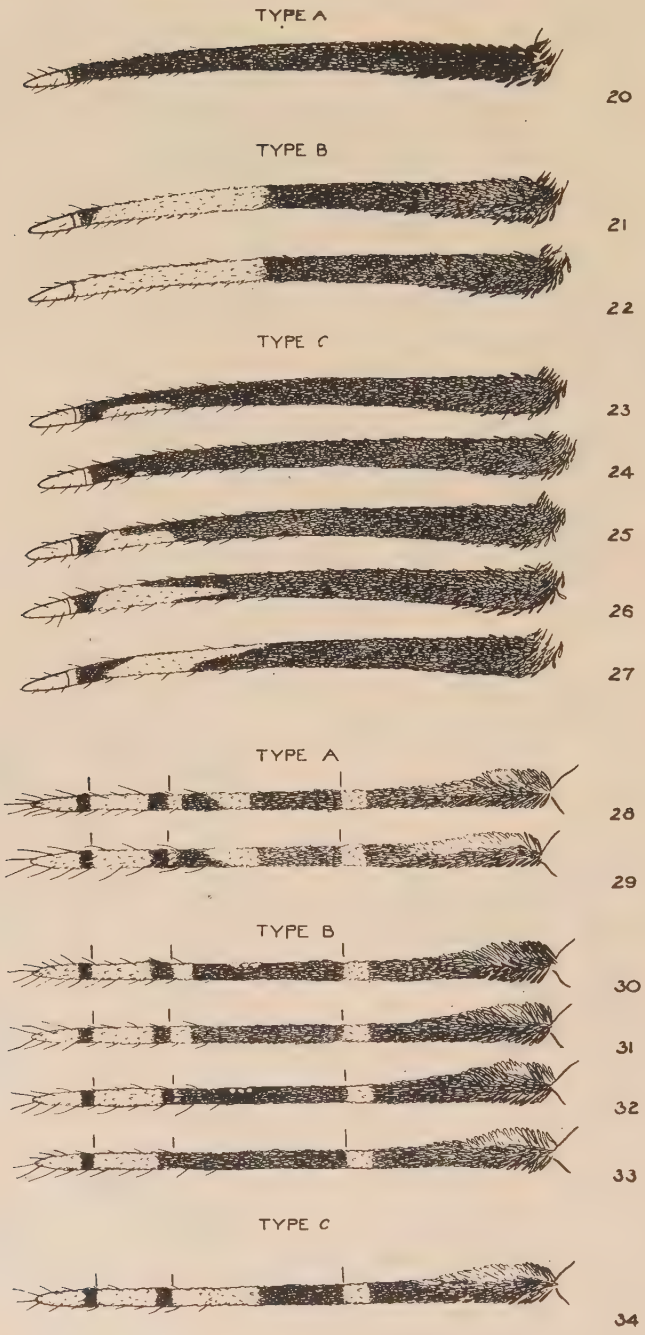
TYPE A
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PLATE VI

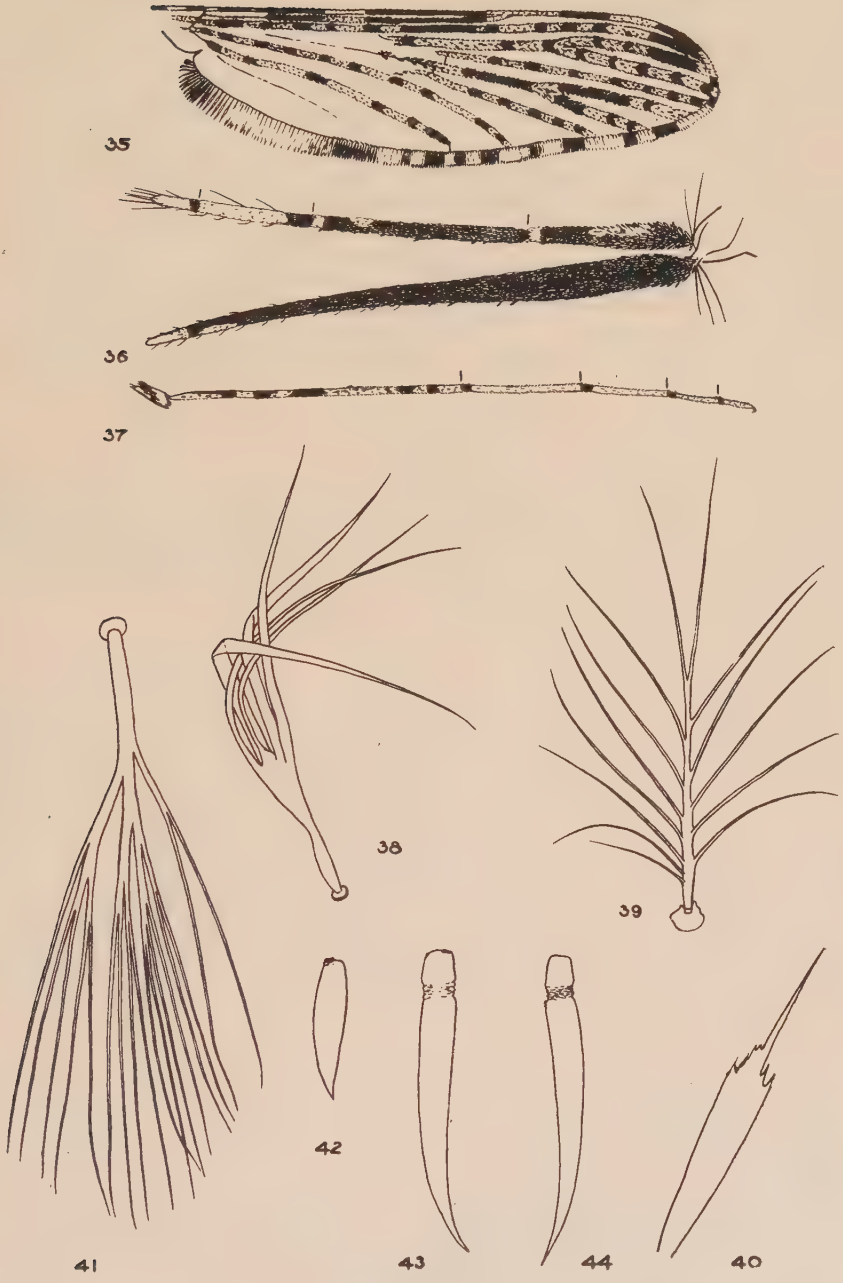


PLATE VII

A NEW GENUS AND SPECIES OF CHIGGER, *CHATIA SETOSA* (TROMBICULIDAE, ACARINA) FROM NORTH-WESTERN UNITED STATES[1]

JAMES M. BRENNAN
Associate Entomologist, United States Public Health Service

The exigencies of the war have stimulated studies of trombiculid mites, several species of which are known transmitting agents of tsutsugamushi disease in many parts of the world.

Incidental to studies of their biology now in progress at the Rocky Mountain Laboratory, hundreds of host animals have been examined and thousands of larvae have been collected.

A new genus and species is described in this paper and data on its host relationships are reported.

Chatia, new genus

Eyes present. Chelicerae small with an inconspicuous subapical dorsal tooth. Pseudochelicerae present. Palpi angulate, the claw terminating in seven or more prongs. All tarsi with only two claws (the claw-like empodium lacking). Coxa III with an irregular row of five to seven setae. Scutum with three pairs of setae similar in form: anteromedians, anterolaterals, and posterolaterals; no anterior median process or crista; sensillae flagelliform. A definite constriction is present just posterior to coxae III, seen best *in situ* and most pronounced in well engorged specimens.

Genotype: *Chatia setosa,* new species.

This distinctive genus is named in honor of my wife who assisted in collecting.

Because of the absence of a third tarsal claw, *Chatia* would fall in the subfamily HEMITROMBICULINAE, established by Ewing (1944) for his monotypical genus *Hemitrombicula*. However, no further consequential morphological affinities exist between these two genera. Furthermore, phylogenetic and biological relationships seem remote. Superficially, *Chatia* resembles *Comatacarus* Ewing, from which it is immediately separated by the tarsal claws, palpal claw, scutum and other characters. The genus is unique, for it exhibits no close affinities with any of the genera of the family TROMBICULIDAE. But, until more evidence is available, the erection of a subfamily to receive *Chatia* is neither desirable nor necessary at this time.

Chatia setosa, n. sp.

Facies (in situ): A large species. Opaque white; hairy; oval, but with a well-defined constriction just posterior to coxae III, best seen in engorged specimens.

Size: Derived from measurements (in microns) of 30 larvae in varying degrees of engorgement. Length, including capitulum: maximum 1186, minimum 374; width: maximum 806, minimum 250.

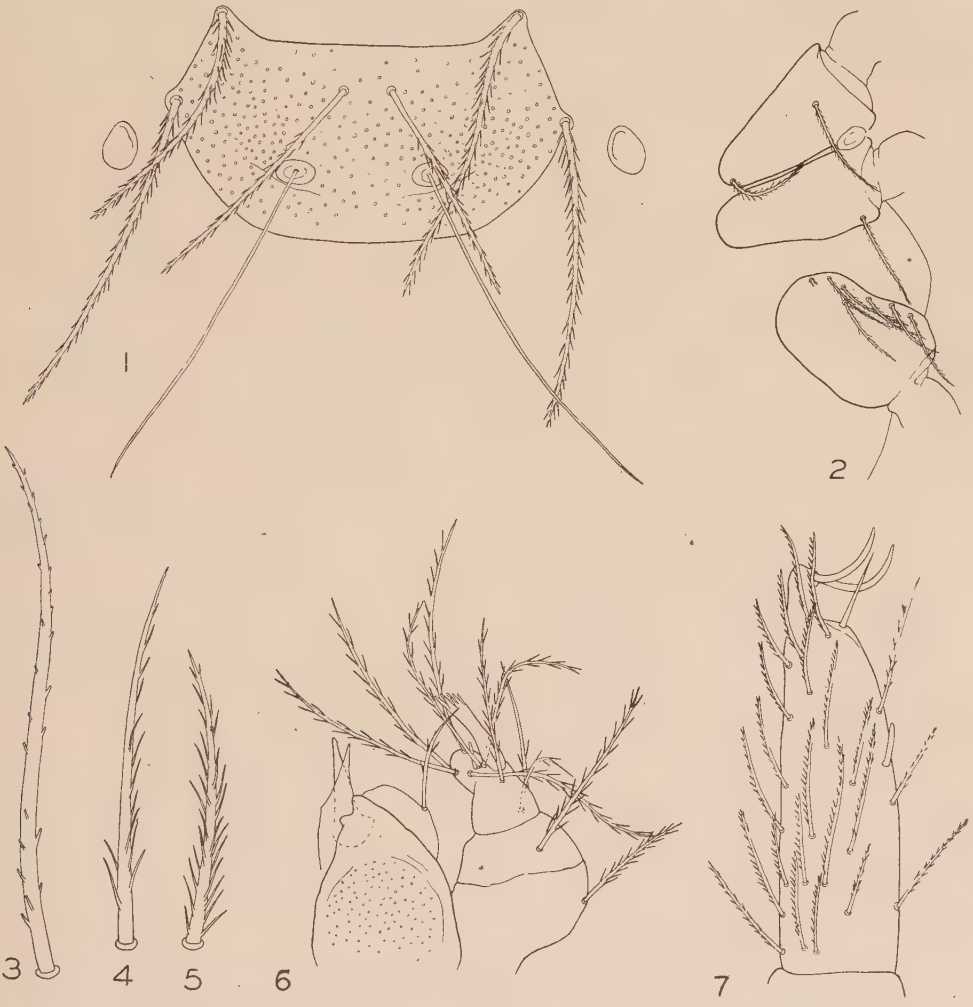
Capitulum (Fig. 6): Chelicerae small, the subapical dorsal tooth inconspicuous; pseudochelicerae long, sharp, but lightly sclerotized and approximating the size of the chelicerae; base of chelicera longer than wide, punctate on basal half, with notched inner distal margin and broadly curved outer margin; cheliceral shield with an outer distal, sparsely branched seta. Palpi broad, subangulate; the thumb prominent, about as broad as long, possessing five long plumose setae and a short spine; claw slender, terminated by a cluster of seven or more short prongs; palpal setae as follows: 1 and 2 plumose, 2 longer than 1, 3 and 4 shorter than 1 and with few branches, 5 about as long as 2 and moderately plumose.

Received for publication, January 10, 1946.

[1] From the Rocky Mountain Laboratory (Hamilton, Mont.), National Institute of Health.

Scutum (Fig. 1): Large, minutely punctate, broader than long, the anterior margin nearly straight and the posterior margin broadly convex. All setae thick, strongly plumose; anteromedians, anterolaterals, and posterolaterals increase in length respectively; the anteromedians are removed from the anterior margin approximately the distance between their bases. Sensillae long, flagelliform, simple, their bases separated slightly more than the distance between anterolateral and posterolateral setae and located approximately the same distance from the posterior margin as the anteromedians from the anterior margin.

Standard data (see Womersley and Heaslip, 1943, and Womersley, 1944) for holotype only: AW–80, PW–107, SB–35, ASB–43, PSB–14, AP–32, AM–63, AL–82, PL–92, S–92.



FIGS. 1–7. *Chatia setosa*, n. sp.

1. Scutum and eyes. 2. Arrangement of coxae and coxal setae. 3. Dorsal seta from posterior rows. 4. Dorsal seta from median rows. 5. Dorsal seta from anterior row. 6. Dorsal view of capitulum. 7. Tarsus I.

Dorsum: A single large eye (Fig. 1) with well-defined cornea on each side of the scutum, near and slightly posterior to the posterolateral corners. Dorsal setae numerous, 160 to 190, variable in form (Figs. 3–5); plumose to barbate, arranged in irregular rows, increasing in length caudad, not so thick as the setae of the scutum, but in the posterior rows considerably longer.

Venter: Setae plumose, about as numerous as the dorsal setae but more uniform in size and structure. Anus situated about midway from coxae III to posterior margin of abdomen.

Legs (Figs. 2, 7): All tarsi with two equal claws, the middle "claw" or claw-like empodium absent. Dorsal spine on tarsus I less than half the length of the actual spine and situated about midway between apex and base; tarsus II with dorsal spine but no actual spine; both dorsal and actual spines absent on tarsus III. Coxa I with one spine, coxa II with one spur and coxa III with a variable number of five to seven spurs in an irregular row near the anterior margin.

Type data.—Holotype larva ex *Tamiasciurus hudsonicus richardsonii*, Bloggett Canyon, Ravalli County, Montana, June 5, 1945, AP–21728.

Paratypes, nine larvae, same data as holotype; seven larvae, same host, same locality, July 11, 1945, AP–21870; seven larvae, same host, Skalkaho Creek, Ravalli County, Montana, Oct. 11 and Nov. 16, 1944, AP–21200 and AP–21201; eight larvae ex *Castilleja lutescens* Rydb., Skalkaho Creek, Ravalli County, Montana, May 10, 1945, AP–21800; one larva ex *C. L. luteorum*, Lolo Canyon, Ravalli County, Montana, June 12, 1945, AP–21735; four larvae ex *C. l. luteorum*, Falls Creek, Elmore County, Idaho, June 26, 1945, AP–21862; one larva ex *Ochotona princeps princeps*, Hughes Creek, Ravalli County, Montana, June 16, 1945, AP–21740; one larva, same host, Ross' Hole, Ravalli County, Montana, Oct. 8, 1945, AP–22018; five larvae ex *Peromyscus maniculatus sonoriensis*, Elmore County, Idaho, June 23–25, 1945, AP–21955; one larva ex *P. maniculatus austerus*, Ravalli County, Montana, Aug. 22, 1945, AP–21969; five larvae, same host, Ross' Hole, Ravalli County, Montana, Oct. 17 and 18, 1945, AP–22041 and AP–22046; two larvae, same host, Gird Creek, Ravalli County, Montana, Oct. 27, 1945, AP–22056; 12 larvae ex *Microtus montanus almicus*, Ross' Hole, Ravalli County, Montana, Oct. 12, 16, and 19, 1945, AP–22034, AP–22039, and AP–22049.

All collections made by personnel of the Rocky Mountain Laboratory.

Holotype and 41 paratypes deposited in the collection of the Rocky Mountain Laboratory; 10 paratypes in the U. S. National Museum; 6 paratypes in the South Australian Museum, and 6 paratypes in the British Museum.

Host data.—Collecting data indicate that *Dermacentor* n. sp. is a mammalian parasite and probably is restricted to the rodents and lagomorphs. The species has not been taken from birds, reptiles or amphibians. From the accompanying information it is indicated further that this dagger does not occur abundantly.

Specimens were found on only six of nearly 300 pine squirrels (*Tamiasciurus hudsonicus richardsoni*) examined, on four of 65 mantled ground squirrels (*Callospermophilus lateralis townsendi*), on five of 154 white-footed mice (*Peromyscus maniculatus* subsp.), and on three of 26 wood rats (*Neotoma cinerea cinerea*); one specimen only from approximately 50 woodchucks (*Marmota flaviventer monacha*), and two specimens from 19 pikas (*Ochotona princeps* subsp.).

The largest number of specimens taken from one animal was approximately 80. Unfortunately this record (*D. n.* [redacted], Ravalli County, Montana, July 11, 1922, AP–1339) cannot be wholly verified, for only two specimens are now available for identification, but from the collector's descriptive notes it is apparent that all specimens of this lot could have been no other than *D. venusta*.

Usually, no specific region of the host is attacked by this dagger, but some preference is exhibited for the underparts. The only instance approximating a concentration of larvae was observed on a white-footed mouse from which 18 specimens were removed from the throat region. None were attached elsewhere.

D. albipictus was found frequently in association with species of the genera *Trombicula* and *Euschöngastia*.

Biological note.—The nymph of *C. tetosa* has been reared in the laboratory from larvae engorged in nature. Description of the nymphal stages and rearing data on this and other species will be given in a later report.

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STUDIES ON REACTIONS OF RAT SERUM TO EGGS OF *TRICHOSOMOIDES CRASSICAUDA*, A NEMATODE OF THE URINARY BLADDER*

VIVIAN S. SMITH

Formation of precipitates *in vitro* around nematode larvae in immune serum was first reported by Sarles (1938) for the rat parasite *Nippostrongylus muris*. Subsequently, similar *in vitro* reactions to nematode larvae in immune serum were demonstrated by Otto (1939, 1940) for *Ancylostoma caninum*, Lawler (1940) for *Strongyloides ratti*, and Mauss (1940) and Oliver-Gonzalez (1940) for *Trichinella spiralis*. Roth (1941) observed precipitates around *T. spiralis* larvae in immune sera of humans, rabbits, and guinea pigs. He suggested the use of this test as an alternative to the Bachman precipitin test for the diagnosis of early cases of trichinosis. Sheldon and Groover (1942) incubated larvae of *Necator americanus* in sera of a group of Georgia school children, and reported formation of precipitates around larvae in a number of cases.

Since Thomas (1924) published the life cycle of *Trichosomoides crassicauda* (Bellingham), no studies have been made upon this common nematode of the urinary bladder of Norway rats. Fundamentally, *T. crassicauda* differs from the above parasites in that the embryonated egg, rather than the third-stage larva, is the infective stage. Eggs are infective when they are laid. The larvae hatch after ingestion by a new host, and penetrate the stomach wall. Contact with the blood stream is of relatively long duration, since adult worms rarely appear in the urinary tract in less than seven weeks after infection has occurred. Although Thomas (1924) reported finding egg-laying adults as early as three weeks from the date of infection, a much longer prepatent period was observed in the present study.

MATERIALS AND METHODS

Albino rats used for laboratory studies were bred from a stock that was guaranteed parasite-free by Sprague-Dawley and Co., Madison, Wisconsin. To ensure maintenance of the colony in this condition, animals were kept in a special type of suspended cage with a removable screen bottom, and cages were autoclaved weekly throughout the experimental period. Since autopsy is the only certain means of determining whether an animal is infected with *T. crassicauda*, rats could not be checked before being used in experiments. Rigid precautions were maintained at all times. Although other rats were present in the same room, the experimental colony was cared for entirely separately by the author. A single instance of known accidental infection of a control rat occurred in an early experiment.

Eggs for infection were obtained by autopsy of wild rats killed in the vicinity of Urbana, Illinois. Gravid female worms were removed from the urinary bladder and used whole after mature (brown-shelled) eggs in their uteri had been counted. Occasionally also the bladder was used if many eggs were clinging to its walls. Within the uteri of female worms there are also large numbers of thin-shelled eggs

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containing larvae which appear quite fully developed. However, since only brown-shelled eggs are laid, it is tentatively assumed that only these are actually infective. In serum cultures, larvae hatched from both types of eggs.

Collection of ova from urine of infected animals was found impractical, since the eggs could not be obtained in sufficient numbers. As a means of determining presence of worms in individual rats, urine examinations were unsatisfactory in that they failed to indicate light infections.

Infecting doses of eggs were fed to rats in small meat pellets, each animal being isolated and observed until it was certain that the meat had been consumed. Initial infections were given shortly after weaning.

EXPERIMENTAL DATA

Results of repeated infections.—Four groups of rats were given repeated infections with *T. crassicauda* to determine whether any evidence of acquired immunity could be obtained. Each group consisted of a litter split to furnish experimental and control animals. Rats 1–3 (Group I) received 11 infections of 25–50 eggs over a period of 14 weeks. Rats 4–7 (Group II) received nine similar-sized infections over a period of nine weeks. Rats 8–11 (Group III) received nine infections of 25–100 eggs over a period of 14 weeks. Rats 12–15 (Group IV) received five infections of 100 eggs over a period of 10 weeks. Intervals between infections were not constant because timing as well as quantity had to depend upon availability of infective material from wild rats. Controls on rats 12–15 differed in that they received a single infection of 100 eggs, whereas other control groups were kept uninfected.

Autopsy results on the above animals showed that no piling up of infections occurred, since three worms were the most found in any infected host. Average number of worms in each of the four groups was as follows: 2.7 in rats 1–3, 1.8 in rats 4–7, 0.25 in rats 8–11, and 1.8 in rats 12–15. Three out of four rats of group 8–11 had no parasites, even though each of these animals had received nine infections. The three last infections were of 100 eggs, or twice the size of any infection given in the two preceding groups. Possibly these larger infections brought into action some factor which either prevented arrival of worms in the urinary tracts or caused rapid elimination of parasites harbored there. This factor apparently had not yet come into operation in the case of rats 12–15, which had received only five infections, although all were of 100 eggs apiece. The data were suggestive of an acquired immunity, but no conclusions can be drawn with such small numbers of worms. No parasites were found in any control rats, including the group which had received an infection of 100 eggs each. This last group was autopsied just six weeks from the date of infection, and the parasites probably would not have had time to reach the urintary tract. More will be said about these animals in the succeeding section.

In vitro tests with eggs in serum.—Blood was collected by heart puncture from each of the above rats just prior to autopsy, and stored overnight in the refrigerator to clot. Cultures of *T. crassicauda* eggs in serum were prepared in the following manner: Worms were teased apart in saline on a glass slide, freeing the ova which were then transferred into a sterile 15-cc centrifuge tube and washed in five changes of sterile saline. A ring of sterile vaseline was prepared on a slide, one drop of egg suspension added to one drop of sterile serum, and the preparation sealed with

a coverglass. Bacterial contamination was generally held down to a point where it did not interfere with the eggs and larvae during an observation period lasting 10 to 15 days. Cultures were incubated at 37° C. The chief controls consisted of the cultures in serum from the littermate control rats of each infected group. At the same time that cultures on each litter were prepared a second control using pure saline instead of serum was used. Slides were examined twice daily for the first two days and then once daily thereafter.

The most striking fact observed in cultures was that precipitates appeared around embryonated eggs in sera of infected rats, as shown in Figs. 1–3. Larvae, which hatched out within one to three days from many eggs, were unaffected by flocculums of any kind. Precipitates were generally present only around embryonated eggs which contained active fully-developed larvae, and occurred much more often and in greater quantity around transparent shelled eggs than around mature eggs with thickened brown shells. Although both types of eggs are present in the uteri of female worms, ova are not passed until the shell is thickened.

A variable number of embryonated eggs showed precipitates. It was common to see two eggs near each other almost exactly alike in appearance, one with precipitate material and the other without. Thin-shelled eggs were frequently much distorted when surrounded by flocculum. See Fig. 3. No breaks in the shells could be seen in typical affected ova.

TABLE 1.—*Reaction of rat serum to* T. crassicauda *eggs*

Animals	No. of infections	Eggs per infection	Earliest circum-oval precipitates	Amt. of precipitate
Rats 1–3	11	25–50	24 hours	+++
Controls	0	0	None	–
Rats 4–7	9	25–50	12 hours	+++
Controls	0	0	None	–
Rats 8–11	9	25–100	8 hours	++++
Controls	0	0	None	–
Rats 12–15	5	100	8 hours	++++
Controls	1	100	48 hours	+

Amount of precipitate as indicated in Table 1 (one to four plus) was an arbitrary designation based both on volume of precipitate and number of eggs affected. Eggs in sera from rats 12–15 showed very marked precipitates despite the fact that these animals had received the fewest infections. However, infective feedings were largest (100 eggs) in this group. It should be noted that controls on rats 12–15 showed light precipitate formation which did not appear until 48 hours had elapsed. These animals were bled six weeks after a single feeding of 100 eggs. Autopsy at the same time indicated that adult worms had not yet arrived in the urinary tract.

No precipitate formation occurred around eggs in sera of rats which had never been infected with *T. crassicauda,* nor in saline control cultures.

Observations on larvae in cultures.—Moving larvae were in some instances seen within unhatched eggs up to the fifth or sixth day of observation. A number of eggs hatched in the majority of serum cultures, including cultures from both infected and control rats. Larvae hatched from thin-shelled (immature) as well as thick-shelled (mature) eggs. After two or three days of considerable activity, free larvae gradually became sluggish. For about three days prior to death they appeared greatly vacuolated. In a few cultures, numerous larvae were seen actively pene-

trating the vaseline seal with the aid of the stylet. This suggests the early pattern of behavior in the body. No development was observed. Larvae in most cultures began to die off at about the sixth day; few surviving 10 to 12 days. No precipitates of any kind were formed around living larvae. See Fig. 4. No differences were observed in behavior or survival of free larvae in cultures from infected and uninfected rats.

Signs of life were generally gone from saline control cultures after the first hour of incubation. Cultures in urine were prepared for rats 1–3 and their controls, but life persisted no longer than in saline controls. After this initial attempt, urine cultures were not used.

Lengths of 112 larvae were measured, including a few dead specimens in saline controls. Wide variations occurred within single cultures, and lengths ranged from 244 to 388 μ with a mean length of 308 ± 3.2 μ. Tabulation of measurements failed to show two distinct groupings which might be considered as representing the two sexes.

There were ten females and five males among the infected animals, with corresponding distribution among the controls. Sex was not a factor in the serum reactions.

DISCUSSION

No satisfactory means could be devised of measuring an infection curve during repeated infections with *T. crassicauda,* without autopsying the hosts. Numbers of worms recovered at autopsy were too few to enable the drawing of any conclusions about acquired resistance from repeated infection. Evidence obtained from *in vitro* tests with eggs in serum was much more satisfactory.

The author considers precipitate formation around eggs in "immune" serum to be an antigen-antibody reaction. Time of appearance and volume of precipitates appear to indicate degree of resistance far more reliably than autopsy results. However, circum-oval precipitates are far more difficult to explain than the circum-larval type. Eggs as such do not enter the blood stream, and one cannot conceive of them acting directly as antigens. A possible explanation is suggested by the work of Oliver-Gonzalez (1941). He found separate antibodies against adults and larvae in the serum of rabbits as a result of infection with *Trichinella spiralis.* Larvae in the uterus proved antigenically similar to adults, and became differentiated to the antigenic composition of mature larvae in 20 days. Applying this information to *Trichosomoides crassicauda,* perhaps the embryonated ova are antigenically similar to adult worms. Thus in the rat host, it might be considered that antibody formation only commences when the larvae reach a stage of development where they are antigenically adult. Future experiments will test this hypothesis. That adult worms need not have reached the urinary tract for antibodies to be detectable in serum is indicated by the once-infected controls on rats 12–15. Factors to be explained are relative scarcity of precipitates around brown-shelled eggs and absence of precipitates around eggs where the larvae were not both active and fully developed. Again, not all eggs of the typically affected type showed precipitates.

Absence of development of *T. crassicauda* larvae in rat serum was not surprising in view of the fact that Thomas (1924) reported little development even in the rat host during the first nine days after infection.

If the reaction of serum vs. ova is found to be applicable to other helminth parasites, it opens wide possibilities in the field of diagnosis. For instance, the serum culture method might prove of value in early diagnosis of human schistosomiasis. Very light infections of *T. crassicauda* were successsfully picked up by the serum reaction, as well as infections in which the adult worms had not yet reached their final habitat in the host. Since antibodies in the blood stream generally persist for a period of time after an infection has run out, the serum reaction could also within limits determine whether an animal had been previously infected. This could be of considerable value in experimental studies.

SUMMARY

1. Repeated infections with *Trichosomoides crassicauda* (Bellingham) gave autopsy results suggestive of induction of an acquired resistance, but numbers of worms recovered were too few to warrant definite conclusions.

2. Cultures of *T. crassicauda* eggs in sera of repeatedly infected rats showed a marked contrast to cultures of eggs in sera of parasite-free rats. Precipitates developed around numerous embryonated eggs incubated in resistant sera. No precipitates appeared around eggs in normal sera.

3. Larvae which hatched out of eggs in serum cultures were unaffected by precipitates.

4. A possible partial explanation of the reaction of resistant serum to eggs is suggested, but must be tested in future experiments. The reaction is believed to be of antigen-antibody nature.

5. Application of the reaction of serum vs. ova to other helminth parasites may open wide possibilities in the field of diagnosis.

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EXPLANATION OF PLATE

Eggs and larvae shown were incubated in "immune" rat serum for five days. All but Fig. 4 are magnified ×300. Fig. 4 is an enlargement, giving a final magnification of about ×900.

FIG. 1. Mature brown-shelled egg with slight precipitate in surrounding serum.

FIG. 2. Embryonated thin-shelled egg with masses of surrounding precipitate.

FIG. 3. Embryonated thin-shelled egg with surrounding precipitate, showing distortion of egg shell.

FIG. 4. Living larva in rat serum. Stylet visible at anterior end.

ARE VESICAL CALCULI ASSOCIATED WITH TRICHOSOMOIDES CRASSICAUDA, THE COMMON BLADDER NEMATODE OF RATS?*

VIVIAN S. SMITH

An unusual type of soft calculus occurs with comparative frequency in the urinary bladders of Norway rats. Few reports of such calculi are to be found in the literature. Walter (1866) described them as large masses resembling cooked rice grains, and identified fine "fibers" which floated from the masses in a fluid medium as *Trichosomoides crassicauda* (Bellingham). Thomas (1924) observed them under the erroneous impression that they were papillomas caused by *T. crassicauda,* as reported by Lowenstein (1910). They were described, without any association being made with nematode parasites, in a 1910 report of the U. S. Public Health Service dealing with the rat in relation to public health.

The common host of the bladder nematode, *T. crassicauda,* in nature is *Rattus norvegicus,* the Norway or brown rat, although it has been reported in very low incidence from other species of wild rats. Studies from a variety of locations indicate that the parasite is probably world-wide in distribution. The direct cycle (Thomas, 1924) and lack of necessity for a developmental period of the eggs outside of the rat host have undoubtedly facilitated its spread. Reports of the incidence of *T. crassicauda* in wild rats have been made by Rayer (1843), Lowenstein (1910), Johnson (1918), Balfour (1922), Thomas (1924), and Luttermoser (1936).

In connection with laboratory experiments with *T. crassicauda,* it was necessary for the present author to examine large numbers of wild Norway rats to obtain fresh worm material. Records were kept of the number of these nematodes in each rat, and of the presence or absence of soft vesical calculi. These records include 370 rats killed in the vicinity of Urbana, Illinois, from June, 1942, through December, 1943. The present paper deals with an analysis of the data obtained from wild rats, supplemented by laboratory experiments conducted in an effort to determine the nature and cause of the calculi.

METHODS

Routine autopsy examination for both wild and laboratory rats consisted of removal of the entire urinary tract (kidneys, ureters, and bladder) into a Petri dish of physiological saline. The bladder and ureters were slit open and examined for worms and pathology under a binocular dissecting microscope. Kidneys were teased apart and similarly examined.

Albino rats used for laboratory studies were bred from a stock that was guaranteed parasite-free by Sprague-Dawley and Co., Madison, Wisconsin. Rigid precautions were exercised to maintain the colony in this condition.

Eggs for laboratory infections were obtained by autopsy of wild rats. Gravid female worms were removed from the urinary bladder and used whole for feedings.

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TABLE 1.—*Summary of* T. crassicauda *infestations in wild rats*

Age group	No. of rats	Per cent parasitized	Average No. of worms
Nursing	12	0.0	0.0
Half-grown	86	11.6	0.5
Young mature	59	81.4	4.1
Adult	201	85.6	4.6
Old	12	91.7	7.3
Total males	179	60.3	3.9
Total females	191	70.2	3.1
Total rats	370	65.4	3.5
Parasitized rats	242	100.0	5.4

Counts were made of the number of mature, brown-shelled, eggs within or adhering to each female.

INCIDENCE OF *T. crassicauda* AND VESICAL CALCULI

In wild rats.—For purposes of records, rats were roughly divided into five "age" groups based on size. This division was purely subjective. Infestations summarized in Table 1 include all worms per host throughout the urinary tract. The average infestation of 3.5 worms per rat checks closely with the figure of 3.4 obtained by Thomas (1924) in a study on rats from the same locality.

Certain differences were evident between infestations in male and female hosts. Maximum numbers of worms per host ran much higher in males. The greatest infestation in a female was recorded as 15 worms. Eight male rats showed greater infestations as follows: 16 worms (twice), 18 (once), 20 (once), 21 (twice), 22 (once), 24 (once), and 30 (once). Statistical analysis of the average number of worms per host in the two sexes showed that the difference between the two means was not significant. No greater infestation could be found on record than that of rat No. 200, an adult male harboring a total of 30 worms. Twenty-three parasites were in the urinary bladder, one in each kidney, four in one ureter and one in the other. Of the total 370 rats, 34 or 9.2 per cent were parasitized in the kidneys or ureters or both.

Table 2 summarizes calculus incidence among wild rats. It will be noted that of a total of 32 rats with vesical calculi, all but two were males. Most calculi were found in adult rats, although three were found in "young mature" males. Usually where worms were present in the urinary bladder with a calculus, they were partially embedded in the matrix, as shown in gross view in Text Fig. 1 and in section in Plate I, Figs. 1 and 2.

In laboratory rats and mice.—Of 18 young rats infected with 50 *T. crassicauda* eggs apiece, mucoid calculi were found in four males at autopsy 15 months after infection. The group was equally divided for the two sexes. A control group of 17 littermates of the above rats, which was kept uninfected, showed no mucoid calculi in either sex at autopsy. Two male rats of another group, raised parasite-free, harbored mucoid calculi of the urinary bladder. Three male rats of a mixed group of 15 which had received numerous *T. crassicauda* infections also had calculi.

TABLE 2.—*Incidence of mucoid vesical calculi in wild rats*

Sex	Total rats	No. of rats with calculi	Per cent of rats with calculi	No. of calculi with worms
Male	179	30	16.8	25
Female	191	2	1.1	2
Total	370	32	8.7	27

Typical calculi were found in the urinary bladders of two male albino mice. Mice have not so far been shown to be able to serve as hosts for *T. crassicauda.* To summarize, mucoid calculi were found in seven male laboratory rats which were or had been infected with *T. crassicauda,* in two male rats which were raised parasite-free, and in two male mice. No mucoid calculi were found in female laboratory animals.

STUDIES ON THE NATURE OF THE VESICAL CALCULI

When freshly removed from the bladder, calculi were white to yellowish in color, extremely varied in shape, but frequently with a "stalk" projecting into the urethra. They measured two to ten millimeters in length, and were of a relatively soft gum-

TEXT-FIG. 1. Mucoid calculi from urinary bladders of wild rats. All but one show embedded worms. Magnified 1.5 times.

rubber consistency. Permitted to dry in air, they became exceedingly hard and brittle and appeared translucent.

A series of tests was employed for the purpose of determining the chemical nature of these peculiar bladder stones. Powder prepared by grinding air-dried calculi failed to respond to any of the criteria for known types of urinary stones (Hawk and Bergeim, 1937). The powder was insoluble in water, alcohol, ether, nitric acid, and hydrochloric acid. It was partly soluble in 10 per cent sodium hydroxide, and gave a distinct positive biuret test. Paraffin sections of calculi failed to respond to von Kossa's silver nitrate stain for calcium. Celloidin sections were stained with mucicarmine. In general the stain appeared red, which is a

positive test for mucus, but it graded into light green in many areas. Meaning of the light green staining is not known at this time. The green areas might possibly be disintegrating mucus.

Histological and operative studies were conducted to determine the biological nature of calculi found in the urinary bladders of rats. Sections prepared by both the paraffin and celloidin methods were stained with Heidenhain's iron alum haematoxylin or Delafield's haematoxylin with eosin. The masses proved to be acellular in nature. Iron alum haematoxylin tended to give a uniform black stain, but Delafield preparations stained much less deeply, with eosin counterstain assuming prominence. Appearance of the material was hyaline vesiculated, quite irregular in shades of staining. Frequently, distorted epithelial cells could be distinguished around the edges of calculi. From the point of view of ease of sectioning, celloidin sections were far more satisfactory and were considered more normal in final appearance. Plate I shows relations of worms to the masses.

Fresh calculus material from wild rats, operatively inserted under the skin and into the urinary bladders of two albino rats, was absorbed or eliminated in both locations. A simple abdominal operation with ether anaesthetization was performed in this and the following tests. Entrance into the bladder was either via a trochar or a very short incision afterwards closed by one stitch.

Various materials were operatively inserted into the urinary bladders of rats to determine whether mucoid calculi could be artifically induced by the presence of any of these substances. Materials inserted were dried mucoid calculus, dead specimens of *T. crassicauda,* human hair, bits of cotton batting, wood shavings, and coal dust. One animal of each sex was used in each case. Autopsies were performed 12 weeks from the date of operation. Although various aberrations of the urinary bladder resulted, such as development of other known types of stones, none of the autopsy results was even suggestive of formation of mucoid calculi.

DISCUSSION

Incidence of parasitism with *T. crassicauda* in wild rats ran very much higher than any infestations produced by the author in laboratory rats. Limited availability of infective material, however, prevented administration of massive infections to laboratory animals. Theoretically, exposure would be almost constant in wild rats, and heavier infestations could be built up. Cannibalism may be a factor in accounting for sporadic very heavy infestations, or perhaps such rats were debilitated by old age, undernourishment, or disease. Male rats are notoriously more belligerent, and if cannibalism can be considered, it may explain why all infestations of over 15 worms occurred in males. No adult parasites were found in nursing rats. This is easily explainable, since the author observed that six to seven weeks had to elapse in laboratory animals between the date of infection and the time of appearance of adult worms in the urinary tract. Rats are weaned at about three weeks of age.

Combining data on incidence of mucoid calculi in laboratory and wild rats we find that all but two cases of a total of 41 occurred in male rats. Although 84.4 per cent of calculi in wild rats were associated with worms, the evidence of two parasite-free laboratory rats must be considered to rule out *T. crassicauda* as a sole cause of calculi, even on the assumption for wild rats that worms could initiate stone formation and then pass out of the bladder. However, further analysis of incidence of calculi in male wild rats shows the following:

Total male rats 179; 16.8% vesical calculi
Infected males 108; 23.1% vesical calculi
Uninfected males 71; 7.0%vesical calculi

This does appear to indicate some relationship between infection in males and the presence of vesical calculi. A second possibility which suggests itself is that male hormones are involved in calculus formation. Carefully controlled experiments are needed to check this factor.

A third possibility is based on the close anatomical association of the seminal vesicles, prostate gland, and urinary bladder of male rats. Both the very large prostate gland and the seminal vesicles have mucoid secretions. Certain conditions might permit an occasional backing up of these secretions through the common urethra into the urinary bladder. A large percentage of calculi have a thin stalk extending into the urethra, and almost all have been found at the urethral end of the bladder. Excess mucus secretion by the transitional epithelium of the bladder, such as might be caused by worms tunneled into the epithelium (see Plate II, Figs. 3 and 4), could further favor calculus formation. Rayer (1843) reported the only observed "damage" to the bladder in *T. crassicauda* infection was presence of more mucus than when worms were absent. In several cases the author has observed stringy masses of material at the urethral end of the bladder, generally in conjunction with worms, which might well be interpreted as incipient mucoid calculi. Laboratory tests seem to rule out mechanical presence of various materials as a cause of mucoid calculi.

Histological studies, as well as subcutaneous and intravesical transplantation of fresh calculus material, ruled out the erroneous assumption by Thomas (1924) that mucoid calculi were identical with the papillomas described by Lowenstein (1910–1913), as being caused by *T. crassicauda*. Worms found by Lowenstein to be associated with tumors in rat urinary tracts may have been incorrectly identified as *T. crassicauda,* and may instead have been a related species which does not occur in the United States. Feldman (1932), in listing types of neoplastic growths found in wild rats in America, makes no mention of tumors of the urinary bladder. Two instances of hyaline necrosis of the bladder wall were seen by the author, in adult male wild rats which each also harbored a mucoid calculus and a number of adult *T. crassicauda.*

SUMMARY

1. Of 370 wild rats, 65.4% were found to harbor *Trichosomoides crassicauda* (Bellingham). Average infestation for the entire group was 3.5 parasites per rat.

2. Peculiar calculi found in the urinary bladders of wild and laboratory rats were found to be acellular and mucoid in nature.

3. All but two of a total of 41 instances of mucoid calculi occurred in male rats. Calculi were much more likely to be found in male rats infected with *T. crassicauda*. However, presence of calculi in the bladders of two male laboratory rats raised parasite-free bars the assumption of *T. crassicauda* as the sole cause.

4. A tentative explanation of the origin of mucoid calculi is proposed: A backflow of the mucoid secretions of the seminal vesicles and prostate gland from the common urethra into the urinary bladder of the male rat might occasionally occur. This nucleus for calculus-formation might be further favored by the ex-

cess mucus secretion caused by *T. crassicauda* tunneled into the bladder epithelium. Necessary accumulation of mucoid material would rarely occur in a female rat.

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Explanation of Plates

Plate I

Sections of mucoid calculi stained with Delafield's haematoxylin and eosin. ×100.

Fig. 1. Paraffin section. Note sections of worms, with eggs taking very heavy stain.

Fig. 2. Celloidin section. Eggs within embedded worm are stained very heavily.

Plate II

Sections of rat urinary bladders showing worms embedded in epithelium. Heidenhain's iron alum haematoxylin. ×200.

Fig. 3. Portion of contracted bladder, with sections of a single coiled worm. Eggs stained very heavily.

Fig. 4. Portion of bladder with a single embedded worm. Eggs stained black.

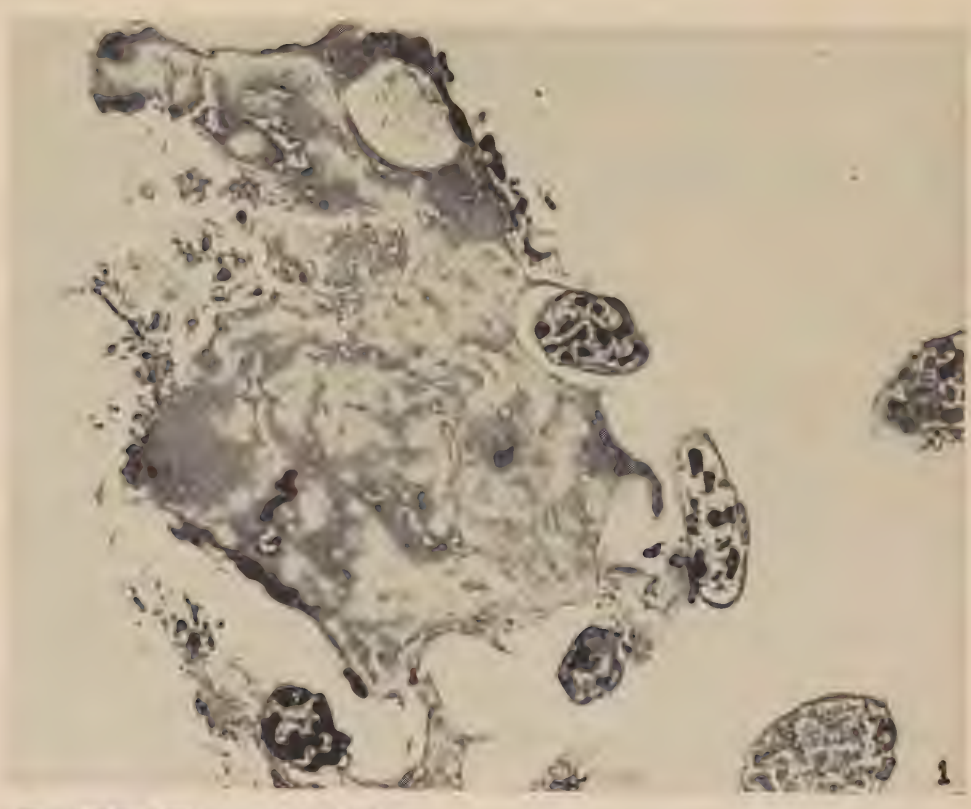

PLATE I

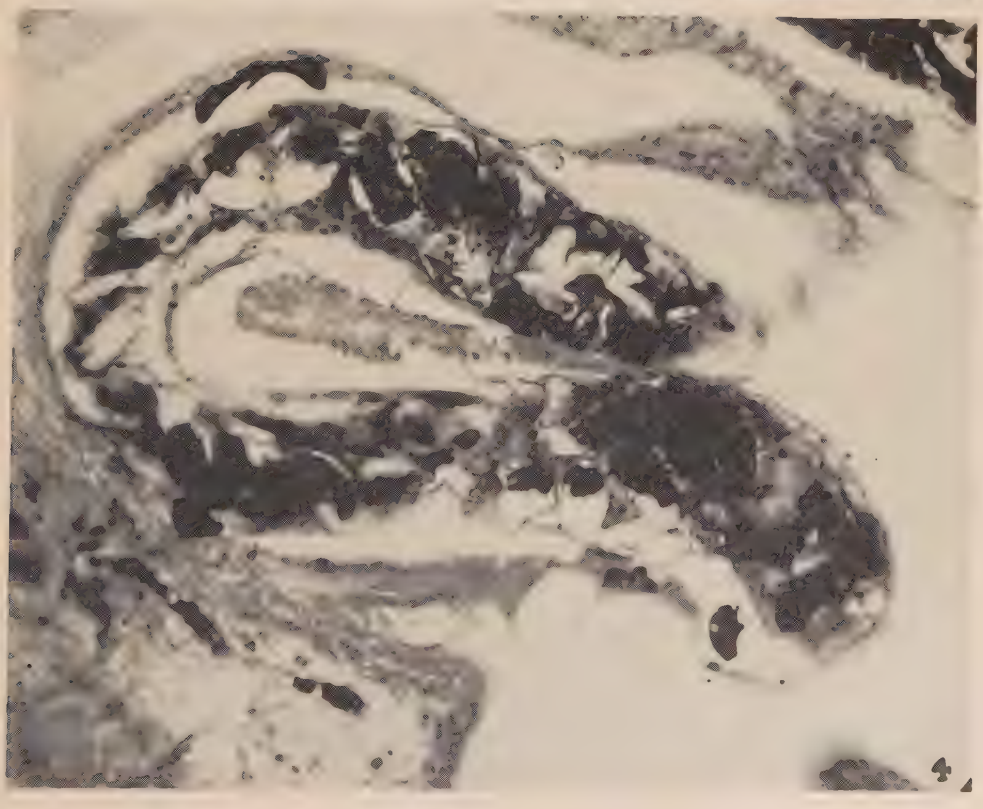

PLATE II

PRELIMINARY NOTES ON THE CRUSTACEAN VECTOR OF THE MAMMALIAN LUNG FLUKE (*PARAGONIMUS*) IN THE PHILIPPINES

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The occurrence of human paragonimiasis in the Philippines was first reported by Musgrave (1907). Since that time additional cases have been encountered, but not all of them have been recorded in the literature, thus creating the impression that the infection is very rare in the Islands. As first suspected by Garrison (1908), the parasitism is not widely distributed, but appears to be confined to certain localities where the appropriate intermediate hosts are undoubtedly present and where the food habits of the people enable the infective stage of the causative agent to be introduced into the human body.

In May and July, 1941, survey trips were made to Naga, Camarines Sur, Luzon, a suspected endemic place, with the object of gathering material for the study of the life history of the fluke. Numerous samples of snails (mostly *Melania* of various species), crabs (*Potamon* (*Potamon*) *sp.*), and prawns (3 species of *Palaemon*) were collected from small creeks near the foot of Mount Isarog. The snails were crushed and examined in the usual way for the presence of *Paragonimus* cercariae and the crustaceans were dissected and the soft parts examined separately between glass slides for the presence of encysted metacercariae. The snails and prawns were negative, but the crabs were found harboring encysted metacercariae which are whitish, globular and double-walled, the inner wall measuring 300 to 320 microns in diameter and the outer wall 340 to 400 microns. Of 216 crabs dissected, 60 per cent were positive, each crab harboring from 3 to 53 cysts. The cysts were most frequently encountered in the muscles of the legs, then in the gills, liver, and thoracic muscles.

When fed to experimental rats and cats, the metacercariae developed into adult lung flukes which are morphologically indistinguishable from adult *Paragonimus* of local human origin. It may be presumed that human paragonimiasis is the result of the ingestion of the same kind of larvae, for it has been learned that river crabs are sometimes eaten uncooked by some of the people. The procedure consists in simply removing the legs, carapace and gills of the live crustaceans and eating the remaining soft parts with vinegar and salt. At other times the crabs are first killed by shaking them with ordinary salt and after 24 hours, during which time partial decomposition takes place, they are ready to be eaten. In order to determine the relation of these methods of eating the crabs to the epidemiology of paragonimiasis, observations were made on the effect of the condiments used on the viability of the cysts. In one experiment, the crabs were shaken with salt and it was noted that the crustaceans were either dead or moribund 7 hours later. When examined at this

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* In a letter transmitted with the three manuscripts, published in this issue of the Journal of Parasitology, Doctor Tubangui stated that the drawings prepared to illustrate these papers were destroyed by fire when the Japanese burned Manila. Among civilians killed at that time were two parasitologists, Doctor C. M. Africa and Doctor L. Leiva. (Horace W. Stunkard, Chairman Editorial Committee.)

stage, the metacercariae located in the gills, liver and thoracic muscles were found to be dead, but those in the muscles of the legs were still alive and infective to experimental animals. After 24 hours, however, no living metacercaria could be found in any part of the body of the crabs. In another experiment, pieces of fresh infected crab meat were immersed for varying lengths of time at room temperature in vinegar which had 4 grams of acetic acid per 100 cc and to which a pinch of salt was added, and in vinegar diluted with an equal amount of tap water and also containing a small amount of salt. It was observed that the metacercariae can survive for 2 to $2\frac{1}{2}$ hours in pure vinegar and for $3\frac{1}{2}$ to 4 hours in dilute vinegar. Beyond these time limits the larvae died; they became more opaque and granular in appearance and failed to infect experimental animals. In control tests, in which infected crab meat was immersed in tap water, the metacercariae remained alive and infective after 4 or more days. The results of these experiments indicate that the practice of salting crabs and keeping them for about 24 hours with the salt before they are eaten is a good preventive measure against paragonimiasis, for it brings about the death of any metacercaria that might be present. On the other hand, the habit of merely soaking uncooked crab meat in vinegar and salt during a meal is dangerous, for the contact between the condiments and the crab tissues is too brief to insure the destruction of *Paragonimus* cysts.

The failure to ascertain thus far the molluscan intermediate host of the parasite may be due to the fact that the examinations have been based mostly on *Melania* snails. The possibility that another type of snail may be involved is suggested by the results of recent studies on the life cycle of *Paragonimus* in the United States and China. It was planned to make an extensive survey on different kinds of snails in the endemic area, but the project had to be laid aside due to the outbreak of the war.

(NOTE: This paper was originally prepared as a longer communication illustrated with a microphotograph of an encysted *Paragonimus* metacercaria and of the crustacean vector. The illustrations were lost when the Bureau of Science was burned by the Japanese in February, 1945.)

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PLAGIORCHOIDES POTAMONIDES (PLAGIORCHIIDAE), A NEW TREMATODE FOUND IN EXPERIMENTAL RATS

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In the course of a study on the life cycle of the mammalian lung fluke (*Paragonimus*), the meat of some fresh-water crabs, *Potamon* (*Potamon*) *sp.*, collected in Naga, Camarines Sur, Luzon, in July, 1941, and found harboring encysted *Paragonimus* metacercariae, was fed to six white laboratory rats. Two of the rats were killed nine weeks after infection and on autopsy they were found to be infected not only with lung flukes but also with adult trematodes which have been identified as belonging to the genus *Plagiorchoides* Olsen, 1937. Control rats that were not given any crab meat remained free of trematode parasites, thus proving that the experimental animals derived their fluke infections from the crustaceans.

Specific diagnosis: Plagiorchoides: Body elongate, attenuated at both ends, 1.7 to 4.6 by 0.65 to 1.9 mm. Cuticle armed with numerous large spines 20 to 23 microns long by 8.5 to 9.0 microns wide at their bases. Oral sucker subterminal, 0.18 to 0.32 mm across; acetabulum in anterior third of body length, about as large as oral sucker, 0.18 to 0.35 mm across. Pharynx well developed, 0.20 to 0.28 by 0.12 to 0.22 mm; esophagus very short, practically absent; intestinal ceca large, reach to posterior end of body.

Testes in middle third of body length, deeply lobed, one obliquely behind the other; anterior testis 0.38 to 0.97 by 0.34 to 0.74, posterior testis 0.40 to 1.05 by 0.35 to 0.63 mm; each testis may be divided into an anterior and a posterior half connected by a narrow central mass and each containing 3 to 4 principal lobes. Cirrus sac well developed, 0.6 to 1.2 by 0.14 to 0.40 mm, curved around right border of acetabulum; its posterior end reaches as far as in front of anterior testis; incloses large seminal vesicle, well-developed pars prostatica and long protrusible cirrus. Seminal vesicle divided into two parts: a narrow, coiled proximal part representing the vas deferens proper and a much dilated distal part constricted at the middle of its length. Terminal portion of cirrus bears prominent wart-like elevations. Common genital pore to one side (left) of median line, about midway between the two suckers.

Ovary lobulated, preequatorial, pretesticular, to one side (right) of median line, behind cirrus sac, 0.20 to 0.62 by 0.15 to 0.42 mm. Shell gland and oötype complex submedian, between ovary and anterior testis. Receptaculum seminis large, oval, to one side (right) of median line, between ovary and posterior testis. Laurer's canal present. Vitellaria follicular, extend ventrally from level of acetabulum and dorsally from level of genital pore to posterior end of body; follicles embrace intestinal ceca and parts of genital glands dorsally and ventrally, with a tendency to become confluent behind posterior testis; vitelline reservoir small, to one side (right) of median line, immediately behind shell gland. Uterus with short irregular coils arranged in descending and ascending series, passes between ovary and anterior testis and between testes themselves, and is confined posteriorly between intestinal ceca; metraterm large and muscular, on left side of acetabulum opposite cirrus sac. Eggs numerous, slightly asymmetrical, light brown, operculated, with a small knoblike protuberance at abopercular end, 35.2 to 42.5 by 24.2 to 26.4 microns; rim of shell thickened and its surface appears pitted.

Excretory system typical of members of the family PLAGIORCHIIDAE: excretory pore posteroterminal; excretory bladder long, Y-shaped, reaches anteriorly to level of ovary where it gives off on each side a short duct which immediately divides into an anterior and a posterior collecting tubule; each collecting tubule divides into three branches, each of which in turn subdivides into three capillaries; each capillary supposedly terminates in a flame cell so that the flame-cell formula is $2[(3+3+3)+(3+3+3)]$.

First intermediate host: Unknown.

Second intermediate host: Fresh-water crab, *Potamon* (*Potamon*) *sp.*

Definitive host: White laboratory rat.

Location in definitive host: Gall bladder and bile ducts.

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Locality of second intermediate host: Naga, Camarines Sur, Luzon.
Type specimens: Burned with the Bureau of Science, February, 1945.

The genus *Plagiorchoides* Olsen, 1937 (= *Neolepoderma* Mehra, 1937) is thus far represented only by its type species, *P. noblei* (Park, 1936). *Plagiorchoides potamonides* can be readily distinguished from the latter by the lobed or lobulated structure of its ovary and testes and by the presence of wart-like elevations on the surface of the cirrus. In *P. noblei* the genital glands are either globular or only indistinctly lobed and the cirrus is covered with minute spines.

Plagiorchoides potamonides should be kept in mind as a potential human parasite in view of the fact that its infective stage is harbored by a fresh-water crab which plays the rôle of vector to the human lung fluke (Tubangui, 1946). Furthermore it has been shown by McMullen (1937) that some plagiorchids are capable of infecting a variety of vertebrate hosts including man. As a matter of fact human infestations with members of this group of trematodes have been reported by Africa and Garcia (1937) and Sandground (1940).

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ON TWO ACANTHOCEPHALA FROM THE PHILIPPINES

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Leiperacanthus gallinarum Bhalerao, 1937

Several mature and immature specimens of this parasite were collected by Dr. P. G. Refuerzo of the College of Veterinary Science, University of the Philippines, from the small intestine of a native chicken. The following description of mature male and female worms is intended to supplement the original description of Bhalerao (1937) which is based on a single female specimen.

Specific diagnosis: Body moderately heavy, cylindrical, slightly attenuated at both ends; anterior region separated from rest of body by a circular depression forming a sort of collar 0.35 by 0.77 mm in size; proximal two-thirds of collar armed with small spines 25 to 30 microns long.

Male, 30 to 50 mm in length by 1.5 to 2.0 mm in maximum diameter; female, 65 to 70 by 1.9 to 2.5 mm. Proboscis subglobular, 0.44 to 0.47 by 0.40 to 0.42 mm in male, 0.57 to 0.60 by 0.47 to 0.54 mm in female, armed with 18 longitudinal rows of 5 hooks each. Hooks 54 to 68 microns long, gradually diminishing in size anteroposteriorly. Proboscis receptacle slightly bent, 1.00 to 1.10 by 0.35 to 0.38 mm in male, 1.15 to 1.35 by 0.48 to 0.65 mm in female, divided into a double-walled anterior portion and a single-walled posterior portion; anterior portion 0.75 to 0.85 mm long in male, 0.93 to 1.03 mm in female. Brain near middle of length of proboscis receptacle. Four submedian proboscideal sacs of variable size and shape present. Lemnisci 6 to 7 mm long, one slightly longer than its fellow, the shorter one with 5 and the other with 7 large nuclei. Male genital organs in posterior third of body length. Testes elongate, 1.8 to 2.5 by 0.60 to 0.70 mm. Cement glands consist of 8 rounded nucleated cells, the anterior four of which are larger than the others. Cement reservoir 1.15 to 1.20 by 0.38 to 0.44 mm. Bursa well developed. Posterior end of female bluntly conical, with genital opening almost terminal. Ova 64.0 to 68.8 by 40.0 to 43.0 microns, with three envelopes; surface of egg shell sculptured.

Host: Gallus gallus domesticus.
Location: Small intestine.
Locality: Manila, Philippines.

The Philippine specimens differ from the Indian worm described by Bhalerao (1937) in having spines in the anterior region of the body and in the number and arrangement of the proboscis hooks. These characters are of diagnostic importance, but it has not been thought advisable to propose a new species, considering that the original description of the Indian parasite is based on a single female specimen. In the future, if on examination of additional material from India the differences noted are constant, then the Philippine form must be separated as a distinct species.

Filisoma rizalinum n. sp.

This parasite is represented by two mature male specimens collected by Mr. A. A. Canonizado of the Bureau of Science from a fish caught in Manila Bay.

Specific diagnosis: Body very slender, cylindrical, without spines, with numerous subcuticular nuclei especially in region of testes, 23.0 to 27.5 mm in length by 0.4 to 0.5 mm in maximum diameter. Proboscis slightly bent, 0.80 to 0.95 by 0.10 to 0.12 mm, armed with 16 longitudinal rows of 23 to 24 hooks each. Two submedian dorsal rows of hooks differ from the other rows in having the first 8 to 10 hooks of each row much larger and less pointed than the other proboscis hooks. These larger hooks are 40 to 50 microns long by 18 to 20 microns wide at the base. The

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hooks of the other rows are larger near middle of length of proboscis, measuring 36 to 40 by 8 to 9 microns, gradually diminishing in length posteriorly to about 16 microns; hooks near anterior end of proboscis 28 to 32 by 6 to 8 microns. Proboscis receptacle double-walled, 1.4 to 1.6 by 0.10 to 0.12 mm. Brain near base of proboscis receptacle, from posterior end of which retinacula emerge. Lemnisci almost as long as receptacle. Male genital organs in posterior half of body. Testes elongate, 1.05 to 1.60 by 0.26 to 0.35 mm. Cement glands consist of 4 very elongated bodies bunched together, containing numerous prominent nuclei and measuring 4.5 to 6.5 mm long.

Host: Fish (*Scatophagus argus*).
Location: Intestine.
Locality: Manila Bay at Malabon, Rizal, Luzon.
Type specimens: Burned with the Bureau of Science, February, 1945.

The genus *Filisoma* Van Cleave, 1928, includes the following three previously described species: *Filisoma indicum* Van Cleave, 1928; *F. bucerium* Van Cleave, 1940; and *F. microcanthi* Harada, 1938. *F. rizalinum* differs from the first two species in having two submedian dorsal longitudinal rows of proboscis hooks, the anterior members of which are conspicuously larger and less pointed than the other proboscis hooks. In *F. indicum* the proboscis hooks are more or less of uniform pattern and in *F. bucerium* only the mid-dorsal longitudinal row of hooks is differentiated. It has not been possible to compare *F. rizalinum* with *F. microcanthi* due to inability to consult the original description of the latter.

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THE TREMATODE GENUS *OPECOELOIDES* AND RELATED GENERA, WITH A DESCRIPTION OF *OPECOELOIDES POLYNEMI* N. SP.*

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This paper is based on thirteen specimens of trematodes, representing two genera, collected in 1941 by Dr. Donald V. Moore from *Polynemus octonemus* Girard, a threadfin fish, in the vicinity of Galveston, Texas. Three of the specimens are identified as *Pseudopecoeloides carangi* (Yamaguti, 1934); the other ten specimens are considered a new species of *Opecoeloides* Odhner, 1928.

The writer wishes to express her appreciation to Dr. H. W. Manter, University of Nebraska, for making available these and other specimens and for many helpful suggestions, and also to Miss Marjorie Jean Raecke, U. S. Bureau of Animal Industry, Washington, D. C., for information regarding the type specimen of *Cymbephallus fimbriatus* Linton, 1934.

THE GENUS *Opecoeloides*

The genus *Opecoeloides* (superfamily: ALLOCREADIOIDEA; family: OPECOELIDAE) was named by Odhner in 1928 for *Distomum furcatum* Bremser, 1819 (in Rudolphi, 1819). This trematode had been rather completely described by Lühe (1900), although he did not include figures of it. The only known reliable figure of *D. furcatum* is by Stossich, 1883, reprinted in Bronn's Thierreichs, Vol. 4, (1893).

The chief generic characters of *Opecoeloides* are: absence of a cirrus sac; presence of an accessory sucker; ceca which open into the excretory vesicle; and a pedunculated acetabulum with papillae. One additional species has been named: *Opecoeloides manteri* (Hunninen and Cable, 1940) Hunninen and Cable, 1941.

A study of trematodes similar to *Opecoeloides* reveals that the exact nature of the ends of the intestinal ceca is often undetermined and that the error of mistaking the accessory sucker for a "genital sucker" has persisted, although corrected by Lühe and by Odhner for *Opecoeloides furcatus*. (Odhner retained the neuter spelling "furcatum." The spelling of the word "Opecoeloides" could indicate either neuter or masculine, but since *Opecoeloides* means "like Opecoelus" and *Opecoelus* is masculine, *Opecoeloides* is here interpreted as being masculine.) A revision of some of these species will be considered later.

Opecoeloides polynemi n. sp.
(Figs. 1–4)

The body is smooth, flattened, and rounded at both ends. It measures 1.706 to 2.289 mm long by 0.182 to 0.381 mm wide. The oral sucker varies from 0.124 to 0.180 mm in transverse diameter. A short prepharynx is present; the pharynx is 0.083 to 0.133 mm long and 0.072 to 0.096 mm wide; when contracted it may be as wide as long. The esophagus measures 0.064 to 0.136 mm, bifurcating immediately anterior to the acetabulum. The ceca open into the excretory vesicle near the excretory pore (Fig. 2).

The acetabulum is stalked. In all ten specimens it is extended so that no measurement of transverse diameter could be taken. The longitudinal diameter of the extended acetabulum ex-

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clusive of stalk measures 0.158 to 0.194 mm; its depth 0.158 to 0.237 mm. The outer rim of the acetabulum is divided into an anterior lip, a posterior lip, and two bilobed lateral lips. Each of the lips possesses a definite number of prominent finger-like papillae. The anterior and posterior lips have two papillae each. The lateral lips bear three papillae, a pair and a single, either of which may be anteriorly placed on the lip. When the pair is anterior on the left lip, the single is anterior on the right lip. The converse also occurs, but the single papilla is always laterally opposite a pair on the other lateral lip. Short, longitudinal muscles occur in each lateral lip near the juncture of the two lobes (Fig. 3).

The accessory sucker is located on a level with and to the left of the esophagus. It is surrounded by a distinct limiting membrane. Its transverse diameter is 0.038 to 0.064 mm.

The genital pore is immediately anterior and slightly to the right of the accessory sucker. The testes are located in the posterior half of the body, are tandem but not touching, and are rounded or slightly lobed. The sinuous seminal vesicle is long, extending to two-thirds the distance between the genital pore and the ovary. The cirrus is short, 24 by 17 μ, and muscular. The pars prostatica, 76 to 96 μ long, is conspicuous and gently curving to the right of the accessory sucker (Fig. 4). The post-testicular space is from 19 to 29% of the total body length, usually 22 or 23%.

The ovary is rounded and pretesticular, lying near the middle of the body. The transverse uterine folds are entirely preovarian. The terminal portion of the uterus lies to the right of the pars prostatica and opens into a common genital pore. Follicular vitellaria fill the lateral fields from near the base of the acetabular stalk to the end of the body. Posterior to the testes they also fill the intercecal region. Four vitelline ducts, two of which serve the posterior part of the body, converge immediately anterior to the ovary. Most of the eggs collapsed in mounting so that measurements may be slightly misleading. What seem to be typical sizes, in utero, are 48 to 54 μ by 24 to 29 μ.

The excretory vesicle is I-shaped, extending anteriorly to the ovary. The excretory pore is subterminal and dorsal.

Measurements in millimeters of the type specimen: *Size:* 2.138 by 0.288. *Oral sucker:* 0.158 by 0.144. *Acetabulum:* Depth 0.216, longitudinal diameter including stalk 0.237, without stalk 0.180. *Sucker ratio of longitudinal measurements:* 1 : 1.25. *Accessory sucker:* Transverse diameter 0.053. *Pharynx:* 0.096 by 0.096. *Esophagus:* 0.100. *Seminal vesicle:* Extends 71% of the distance between genital pore and ovary. *Post-testicular space:* 23% of body length.

Host: Polynemus octonemus Girard, threadfin fish.
Locality: Gulf of Mexico, Galveston, Texas.
Location: Intestine.
Number: Ten from one fish.
Type specimen: U. S. National Museum, Helm. Coll. No. 45887.

A comparison of *O. polynemi* with related trematodes will follow a consideration of the generic status of several species.

Cymbephallus LINTON, 1934, A SYNONYM OF *Opecoeloides* ODHNER, 1928

A study of the genus *Cymbephallus* Linton, 1934 was undertaken because a number of Linton's figures (Linton, 1900, Figs. 38–39; 1901, Figs. 335–336; 1940, Figs. 18–19) suggested the possibility that he had overlooked the similarity between his specimens and *Distomum furcatum.*

The generic name *Cymbephallus* first appeared in Manter, 1934, credited to Linton whose paper appeared later in 1934. The diagnosis given by Manter (1934: 295) was based on earlier descriptions of *D. vitellosum* Linton, 1900, on certain specimens in his own collections, and on the manuscript of Linton's paper then in press.

The type species of *Cymbephallus* is *Distomum vitellosum* Linton, 1900, from the silver hake, *Merluccius bilinearis* (Mitchill) at Woods Hole, Mass. The original description and figures of this species, as well as Linton's generic diagnosis of *Cymbephallus,* did not indicate any accessory sucker except a "genital sucker" and contained no mention of a uroproct. Thus, Manter's concept of the genus was one with which his *Cymbephallus vulgaris,* with a muscular genital atrium, agreed. In-

dication of discrepancy between the two concepts of the genus became evident in 1940 when Linton redescribed and figured his *Cymbephallus vitellosus* and *Cymbephallus fimbriatus*. Here Linton's figures indicate the presence of a distinct male sucker described as: "Genital pores in front of ventral sucker, to left of midline, male aperture surrounded by a strong, muscular, sucker-like structure" (Linton, 1940: 75–76). Another point of uncertainty appeared in the presence or absence of acetabular papillae which were entirely absent in *C. vulgaris* Manter, 1934 but present in some descriptions of *D. vitellosum*. Linton considered them to be too variable to be of taxonomic value.

During the period from 1898 to 1934 Linton collected numerous trematodes which he identified as *D. vitellosum* from more than fifty different hosts. The descriptions are variable and incomplete. The original description of *D. vitellosum* has no mention of papillae on the acetabulum, but a few months later in referring to two small specimens from a flounder, *Paralichthys dentatus* (Linn.), Linton described the posterior edge of the acetabulum as deeply notched so as to form two or three blunt digitate lobes (Linton, 1901: 482, Fig. 336). In 1905, he described a muscular sucker at the male genital opening on one specimen and commented that other specimens had papillae. Later, Linton (1934) stated that the acetabulum is "often more or less pedicellate" and has "4 or 5 lobes on the posterior border and about 4 on the anterior border." The 1940 description of *C. vitellosus* is almost as unsatisfactory as earlier accounts, stating that the acetabulum is more or less prominent or even pedicellate, and has four or five lobes on the posterior border and about four on the anterior border; but two of the three figures (Figs. 18–19) show three anterior and two posterior papillae.

The variability of Linton's descriptions and figures and the uncertain nature of the genus *Cymbephallus* made it evident that a study of the type specimen of *Distomum vitellosum* Linton, 1900 should be undertaken. The specimen (U. S. National Museum Helm. Coll., No. 7060, old number 6351) was loaned by the National Museum through the kindness of Dr. E. W. Price. Since the published data on *Cymbephallus* are conflicting and evidently inadequate, the status of the genus must depend on this type specimen.

The study of this specimen shows that Linton overlooked several important characters. A preacetabular accessory sucker without a limiting membrane is definitely present but it has no connection with the genital ducts (Fig. 5). The acetabulum is retracted within the body wall; hence when extended would be more or less pedicellate. There are three papillae on the anterior lip of the acetabulum and two on the posterior lip (Fig. 6). No anus could be seen, and the ends of the ceca are obscured by vitellaria. The presence of an accessory sucker and of acetabular papillae are characteristic of the genus *Opecoeloides* Odhner, 1928. Furthermore, although the connection of the ceca with the excretory vesicle could not be seen, all other characters and measurements of the specimen agree with those of *Opecoeloides manteri* (Hunninen and Cable, 1940) Hunninen and Cable, 1941. Figures 335 and 336 of Linton, 1901 correspond with figures 6, 8, and 9 of Hunninen and Cable, 1941. The specimen represented in Linton's figure 336 is from *Paralichthys dentatus* (Linn.), one of the hosts recorded by Hunninen and Cable. Figures 18 and 19 of Linton, 1940 clearly show the three anterior and two posterior papillae, which are distinctive for *O. manteri*. Further comparisons were made with a specimen of

O. manteri collected and identified by Cable. Hunninen and Cable made frontal sections to verify the extent of the ceca, which open into the excretory vesicle. It was concluded that the two specimens are of the same species and that, therefore, *Cymbephallus vitellosus* became *Opecoeloides vitellosus* (Linton, 1900) Von Wicklen, 1946 and *Opecoeloides manteri* (Hunninen and Cable, 1940) became a synonym of *O. vitellosus*. This synonymy was reported in abstract by the author (Von Wicklen, 1946). It should be noted that this conclusion regarding *O. vitellosus* refers only to Linton's original material and not necessarily to all of the various collections of trematodes identified by Linton as *Distomum vitellosum* because they almost certainly include a variety of species.

Dr. H. W. Manter kindly lent the author his mounted specimens of *Anisoporus eucinostomi* Manter, 1940. What was originally described as an anus was doubtless a fold in the skin, since sections through the posterior end of a specimen reveal that the ceca open into the excretory vesicle (Fig. 7). This additional information adds another species to the genus *Opecoeloides, Opecoeloides eucinostomi* (Manter, 1940) n. comb. with the synonym *Anisoporus eucinostomi* Manter, 1940.

COMPARISONS

Opecoeloides polynemi is to be compared with three other species in the genus: *O. furcatus, O. vitellosus* (= *O. manteri*), and *O. eucinostomi* (= *Anisoporus eucinostomi*). It can be distinguished from these by the total number of papillae, ten, and by the unique arrangement of the lateral papillae. The accessory sucker of *O. polynemi* is much smaller than that of *O. furcatus* and of *O. eucinostomi,* and differs from that of *O. vitellosus* in having a distinct bordering membrane.

Pseudopecoelus N. GEN.

Cymbephallus vulgaris Manter, 1934 is evidently generically distinct from *Opecoeloides vitellosus*. It has no accessory sucker, no acetabular papillae, and ceca which end blindly. Several other species of *Cymbephallus* similar to *C. vulgaris* have been named. With the fall of *Cymbephallus* into synonymy with *Opecoeloides,* a new generic name is required for these species. The name *Pseudopecoelus* is proposed since this genus resembles *Opecoelus* in that the acetabulum is usually protrusible and the accessory sucker is lacking. It is different in that the ceca end blindly and there are no acetabular papillae.

Generic diagnosis of Pseudopecoelus (OPECOELIDAE): Body smooth, elongated; acetabulum usually protrusible, never papillated; anus lacking, ceca end blindly; muscular genital pore located well anterior to the acetabulum and to the left of the pharynx; testes tandem; cirrus sac greatly reduced or lacking; cirrus short and muscular; prostate gland lacking; seminal vesicle tubular and not enclosed in the cirrus sac; vitellaria from region of acetabulum to the posterior end; ovary pretesticular; uterus preovarian; seminal receptacle lacking; eggs medium-sized to large. Parasites of marine fishes.

Type species: *Pseudopecoelus vulgaris* (Manter, 1934) n. comb.
Synonym: Cymbephallus vulgaris Manter, 1934.

Other species: *Pseudopecoelus japonicus* (Yamaguti, 1938) n. comb.
Synonym: Cymbephallus japonicus Yamaguti, 1938.
Pseudopecoelus elongatus (Yamaguti, 1938) n. comb.
Synonym: Cymbephallus elongatus Yamaguti, 1938.

It is possible that a fourth species is represented by specimens recorded by Hopkins (1941) as *C. fimbriatus* from the white perch, *Bairdiella chrysura*

(Lacépède) at Beaufort, North Carolina. He evidently based his identification on Manter's (1934) interpretation of *Cymbephallus* because, judging from his figures, his specimens have no acetabular papillae, no accessory sucker, and ceca which end blindly. In accordance with the classification above, these specimens would be in the genus *Pseudopecoelus*.

Cymbephallus fimbriatus Linton, 1934 (in Manter, 1934) and later described in Linton (1934) is based on specimens collected by Linton from *Menticirrhus saxatilis* (Bloch and Schneider), kingfish, at Woods Hole, Mass., and identified earlier (Linton, 1901) as *Distomum vitellosum*. It is also recorded (Linton, 1934: 82) from *Bairdiella chrysura, Menticirrhus americanus* and *Sciaenops ocellatus* at Beaufort, N. C., from which hosts he had previously reported it as *D. vitellosum*. He distinguished *C. fimbriatus* from *C. vitellosus* on the basis of larger size, more numerous acetabular papillae, lobed testes, extent of seminal vesicle, extent of vitellaria, and egg size.

As in the case of *C. vitellosus,* the status of *C. fimbriatus* must depend not so much on Linton's early and inadequate descriptions, and obviously not on the specimens identified by Manter (1934: 295), but upon the type specimens from *Menticirrhus saxatilis* at Woods Hole (U. S. National Museum No. 8266). There are nine specimens on two slides. These specimens were studied for the writer by Miss Marjorie Jean Raecke. It seems certain that *C. fimbriatus* cannot be assigned to either *Pseudopecoelus* or to *Opecoeloides* but must represent a new genus.

The *Cymbephallus fimbriatus* Linton, 1934 of Manter, 1934 evidently belongs to the genus *Pseudopecoelus* as described above. Since the characteristics of this latter species do not agree with the type specimens of *C. fimbriatus,* its identification as such was an error, and the species is without a name. The name *Pseudopecoelus tortugae* n. sp. (named for the locality, Tortugas, Fla.) is proposed for it, with the synonym *Cymbephallus fimbriatus* Linton, 1934 of Manter, 1934.

Fimbriatus N. GEN. RELATED TO *Opecoeloides*

A study of specimens of a trematode from *Menticirrhus americanus* (Linn.) collected by Dr. H. W. Manter at Beaufort, N. C., led to the suspicion that the specimens from that host identified by Linton as *C. fimbriatus* could not belong in the genus *Opecoeloides* nor in *Pseudopecoelus*. A report on the type specimens of *C. fimbriatus* by Miss Raecke confirmed this suspicion. An accessory sucker is clearly present with the genital pore lying anterior to it. There are two ani, each cecum opening separately to the outside. The acetabulum is protrusible and has five lobe-like processes, each with a fimbriated border. For this species is proposed the new genus *Fimbriatus*. The genus has the family characteristics of OPECOELIDAE plus the generic distinction of two ani, an accessory sucker, and a pedunculated acetabulum with fimbriated lobes. The type species is *Fimbriatus fimbriatus* (Linton, 1934) n. comb., with the synonym *Cymbephallus fimbriatus* Linton, 1934.

Pseudopecoeloides carangi (YAMAGUTI, 1938) YAMAGUTI, 1940

Among the trematodes from *Polynemus octonemus* were three specimens which resembled *Opecoeloides* except that they had neither an accessory sucker nor papillae on the acetabulum. Yamaguti (1940) created the genus *Pseudopecoeloides* for species with such characters. There are two species in the genus: *P. tenuis*

Yamaguti, 1940 and *P. carangi* (Yamaguti, 1938). *P. carangi* was first described from the Japanese fish *Caranx mertensi* Cuv. et Valenc. and later from *Selar crumenophthalmus* (Bloch) in the region of La Plata Island, Ecuador (Manter, 1940). This report extends the range and host list for *P. carangi.*

The specimens are 2.304 to 2.600 mm long by 0.27 to 0.30 mm wide. The acetabulum, withdrawn in one specimen, measures 0.184 mm in transverse diameter; the oral sucker-acetabulum ratio is 1:1.4. The egg measurements given by Yamaguti are 62 to 72 μ by 33 to 35 μ. Those recorded by Manter are slightly smaller. Measurements in the present study, 67 to 70 μ by 38 to 40 μ are slightly wider than Yamaguti's but fall within his measurements for length. Although not recorded as a generic character, it might be noted that the vitellaria are discontinuous opposite the ovary and the testes in both species.

Comparison of *Opecoeloides* and Related Genera

Genus	*Accessory sucker*	*Acetabulum*	*Acetabular papillae*	*Uroproct*	*Anus*
Opecoeloides	Present	Protrusible	Present	Present	Absent
Pseudopecoeloides	Absent	Protrusible	Absent	Present	Absent
Opecoelus	Absent	Usually protrusible	Present	Absent	One
Pseudopecoelus	Absent	Usually protrusible	Absent	Absent	Absent, ceca end blindly
Anisoporus	Present	Protrusible	Present	Absent	One
Fimbriatus	Present	Protrusible	Present	Absent	Two

SUMMARY

1. A new species of digenetic trematode, *Opecoeloides polynemi,* from *Polynemus octonemus* Girard, a threadfin fish, from the Gulf of Mexico is described.

2. *Distomum vitellosum* Linton, 1900 is redescribed from the type specimen and is found to have a protrusible acetabulum with papillae, and an accessory sucker. Its name now is *Opecoeloides vitellosus* (Linton, 1900). The genus *Cymbephallus* Linton, 1934 is a synonym of *Opecoeloides.*

3. *Opecoeloides manteri* (Hunninen and Cable, 1940) is considered a synonym of *O. vitellosus* (Linton, 1900).

4. *Pseudopecoelus* n. gen. is named for *Cymbephallus vulgaris* Manter, 1934 which becomes *Pseudopecoelus vulgaris* (Manter, 1934) n. comb.

5. *Fimbriatus* n. gen. is named for *Cymbephallus fimbriatus* Linton, 1934 which becomes *Fimbriatus fimbriatus* (Linton, 1934) n. comb. The genus is characterized by fimbriated lobes on the acetabulum, an accessory sucker, and two ani.

6. The *Cymbephallus fimbriatus* Linton, 1934 of Manter, 1934 is given the name *Pseudopecoelus tortugae* n. sp.

7. The following additional changes in nomenclature are proposed: *Opecoeloides eucinostomi* (Manter, 1940) n. comb., synonym: *Anisoporus eucinostomi* Manter, 1940; *Pseudopecoelus japonicus* (Yamaguti, 1938) n. comb., synonym: *Cymbephallus japonicus* Yamaguti, 1938; *Pseudopecoelus elongatus* (Yamaguti, 1938) n. comb., synonym: *Cymbephallus elongatus* Yamaguti, 1938.

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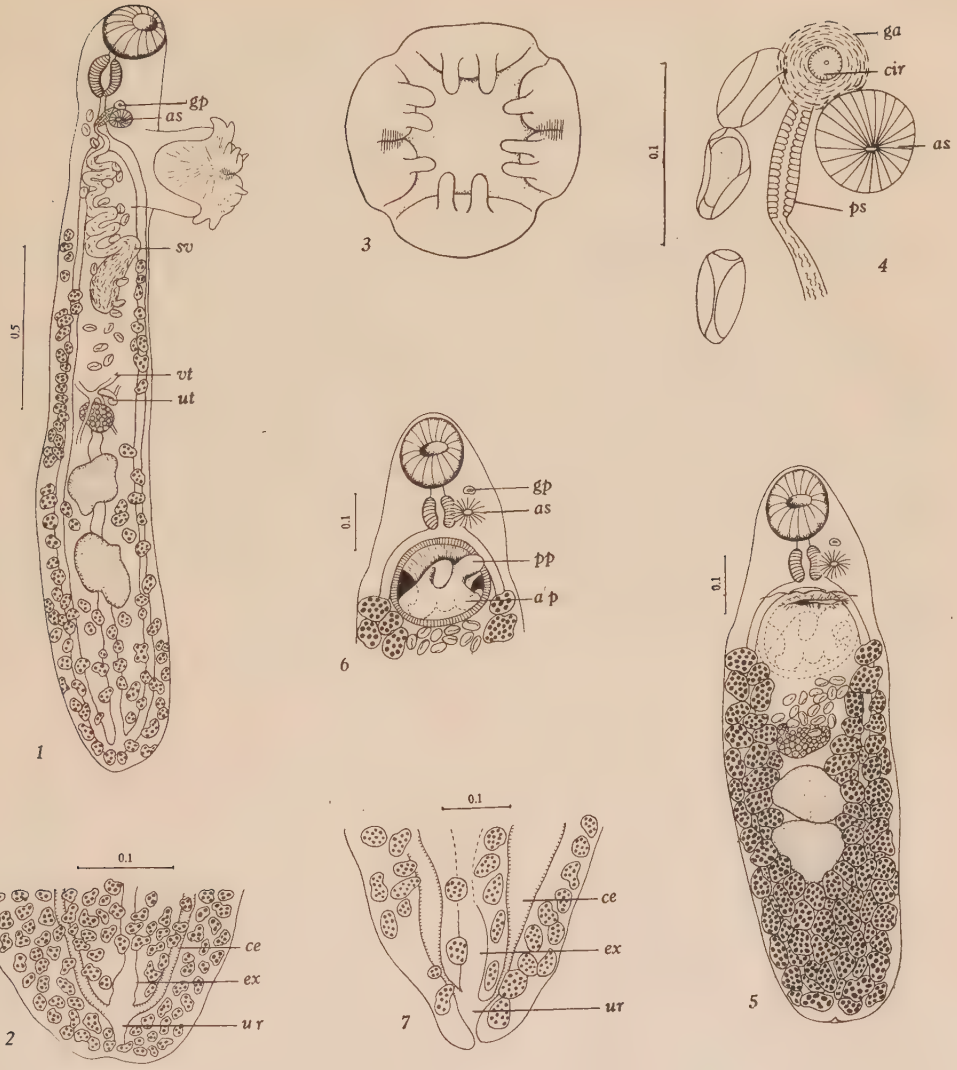

Explanation of Plate

Abbreviations

ap	anterior papilla	*gp*	genital pore
as	accessory sucker	*pp*	posterior papilla
ce	cecum	*ps*	pars prostatica
cir	cirrus	*ur*	uroproct
ex	excretory vesicle	*ut*	uterus
ga	genital atrium	*vt*	vitelline duct

All drawings except Fig. 3 were made with the aid of a camera lucida.

Fig. 1. *Opecoeloides polynemi* n. sp. Ventral view.

Fig. 2. A frontal section through the posterior end of *O. polynemi* demonstrating the union of ceca with excretory vesicle.

Fig. 3. Diagram of a ventral view of an extended acetabulum of *O. polynemi* showing the arrangement and number of papillae.

Fig. 4. Genital region of *O. polynemi.* Ventral view, from a paratype.

Fig. 5. *Opecoeloides vitellosus* (Linton, 1900). Ventral view of type specimen.

Fig. 6. Ventral view of anterior end of *O. vitellosus* with surface of acetabulum exposed to show papillae.

Fig. 7. Frontal section of posterior end of *Opecoeloides eucinostomi* (Manter, 1940), showing uroproct.

ATRICHOLAELAPS SIGMODONI, A NEW SPECIES OF MITE PARASITIC ON THE COTTON RAT, AND NOTES ON THE GENERA *ATRICHOLAELAPS* AND *ISCHNOLAELAPS* (ACARINA: LAELAPTIDAE)*

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During a study of a large series of ectoparasites of the cotton rat, *Sigmodon hispidus texianus,* the mite observed most frequently is the one described herein as new.[1] Laboratory rearing provided many examples of nymphal stages and both sexes of the adult stage. The females are light brown in color and are the form most commonly found on the rat. The males and immatures are whitish and are more readily found in nesting material than on the host. I have never found larvae. This stage is undoubtedly passed within the body of the female. I have many mounted specimens in which the young is clearly visible within the egg, and although many of these are seen to be six-legged larvae, others are certainly the first nymphal stage as evidenced by the presence of four pairs of legs and the short peritreme. Fig. 7 shows two views of the protonymph within the egg. These drawings are from camera-lucida sketches made at high magnification.

Atricholaelaps sigmodoni n. sp.

Protonymph (Fig. 1): Measurements of 10 specimens, exclusive of the mouthparts. Length: average, 425 microns; minimum, 360; maximum, 493. Width: average, 286 microns; minimum, 213; maximum, 360.

Hypostome and chelicerae similar to those of female. Sternal plate indistinct, not reaching the fourth coxa; shaped as figured, with six prominent setae. There is an oblique, slit-like pore below each of the first and second setae. The anal plate is indistinct and with the usual three setae. Between the sternal and anal plates are four pairs of setae, of which the first pair, lying between the fourth pair of coxae is the smallest. The peritreme, about 5 microns long, barely reaches the middle of the third coxa. Dorsally there are two large shields with three pairs of small shields between them. These are arranged as figured and are quite indistinct in mounted specimens. Both the anterior and the posterior shields are bi-concave on their inner margins.

Deutonymph (Fig. 2): Measurements based on 10 specimens, exclusive of mouth parts. Length: average, 477 microns; minimum, 427; maximum, 533. Width: average, 330 microns; minimum, 267; maximum, 349.

Hypostome and chelicerae as in the female. Sternal plate not pigmented but well developed and with traces of transverse lines as in the adult. The plate extends just beyond the fourth coxa and bears four pairs of marginal setae. Metapodal plates are present and as figured. The anal plate shows circular lines and a roughened posterior border as in the female, but lacks pigmentation. The peritreme reaches the first coxa.

The dorsal shield is entire and covers all but a narrow periphery of the dorsal side. It is unpigmented but has the irregular transverse lines similar to the adult. Scattered among the rather long setae are numerous pores.

Male (Figs. 3 and 6).—Measurements based on 10 specimens, exclusive of the mouthparts. Length: average, 461 microns; minimum, 440; maximum, 520. Width: average, 296 microns; minimum, 267; maximum, 347.

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[1] Ectoparasites of the cotton rat were referred to me for identification by Dr. J. Allen Scott during the course of a study of the transmission of filariae.

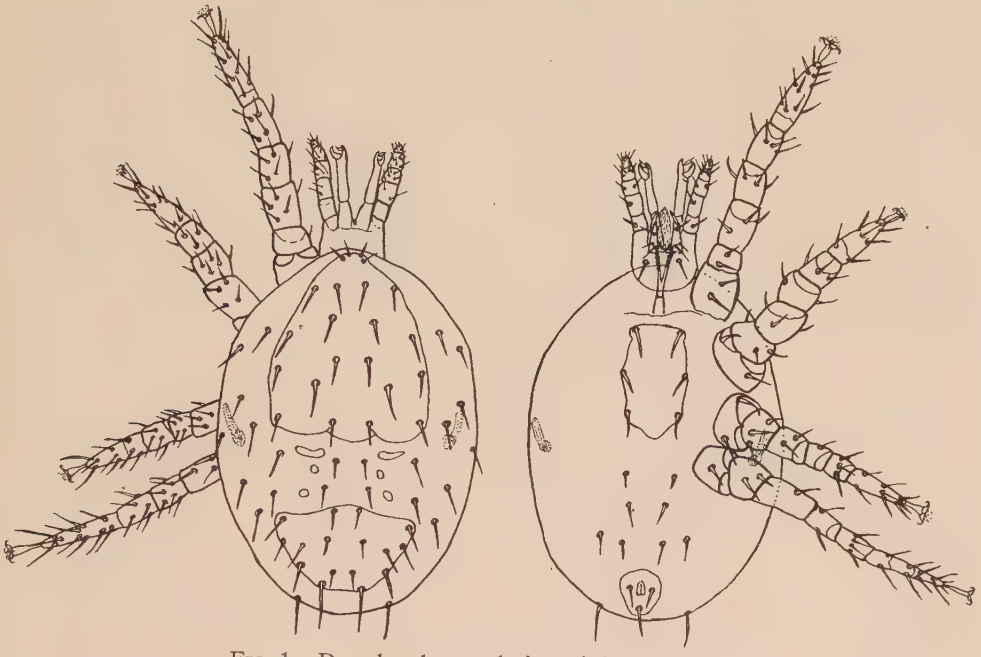

FIG. 1. Dorsal and ventral view of the protonymph.

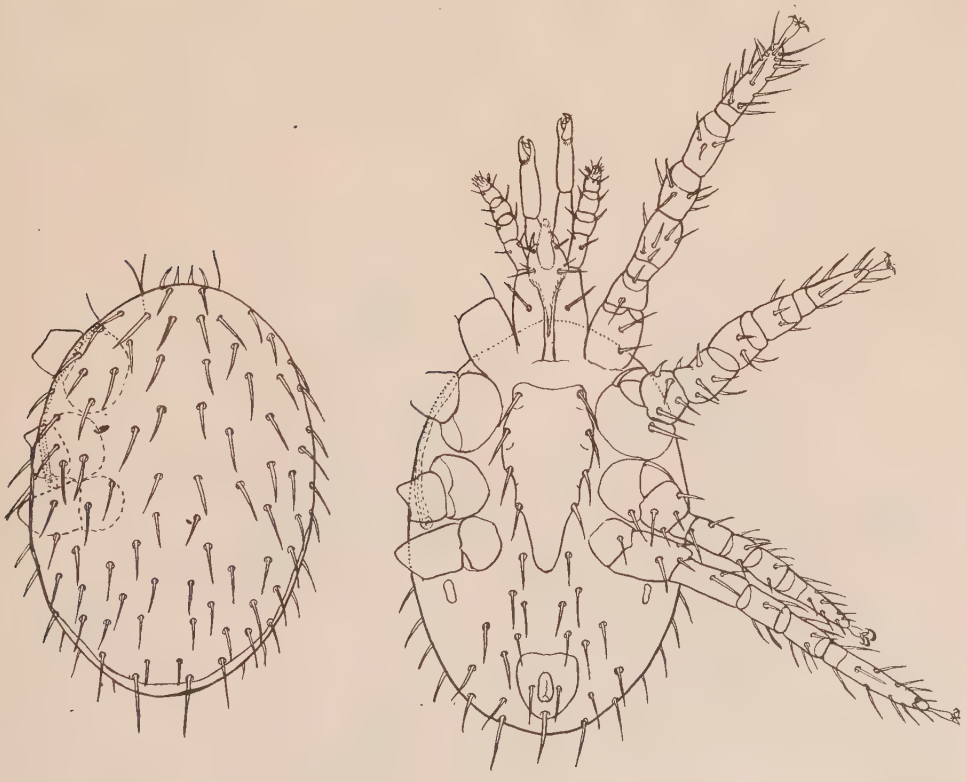

FIG. 2. Dorsal and ventral view of the deutonymph.

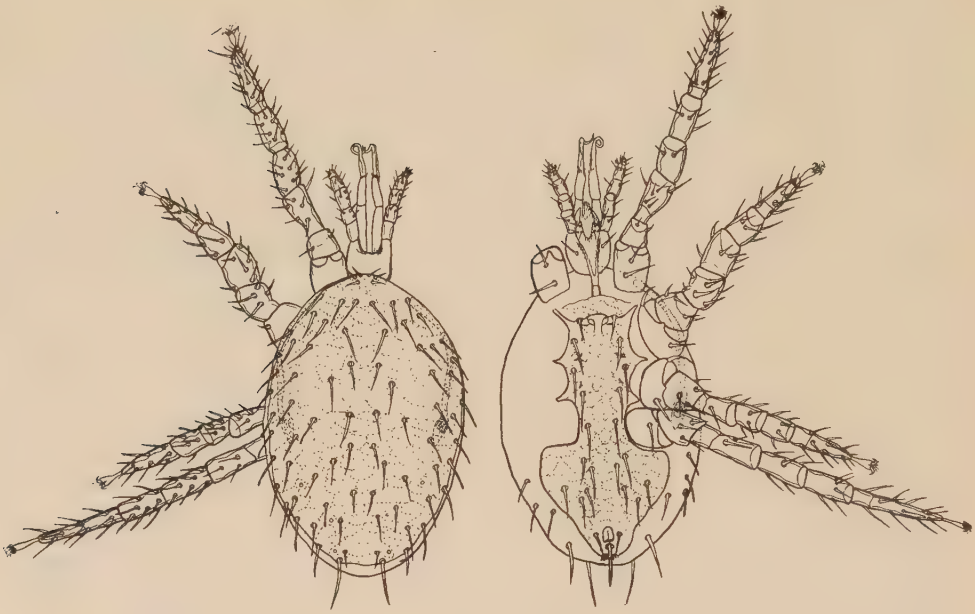

FIG. 3. Dorsal and ventral view of male.

The single chela of each chelicera is long, much longer than the segment that bears it and the apical half, at least in mounted specimens, nearly always makes a complete loop. The ventral plates are united, distinct, with numerous fine lines and 10 pairs of long setae (in addition to the three anal setae), arranged as figured. The genital pore is large and distinct and opens on the anterior border of the ventral plate. On either side of the genital pore is a transverse slit-like pore, between the second and third setae is an oblique pore, and between the third and fourth setae is a short, longitudinal pore. The peritreme reaches nearly to the middle of the first coxa.

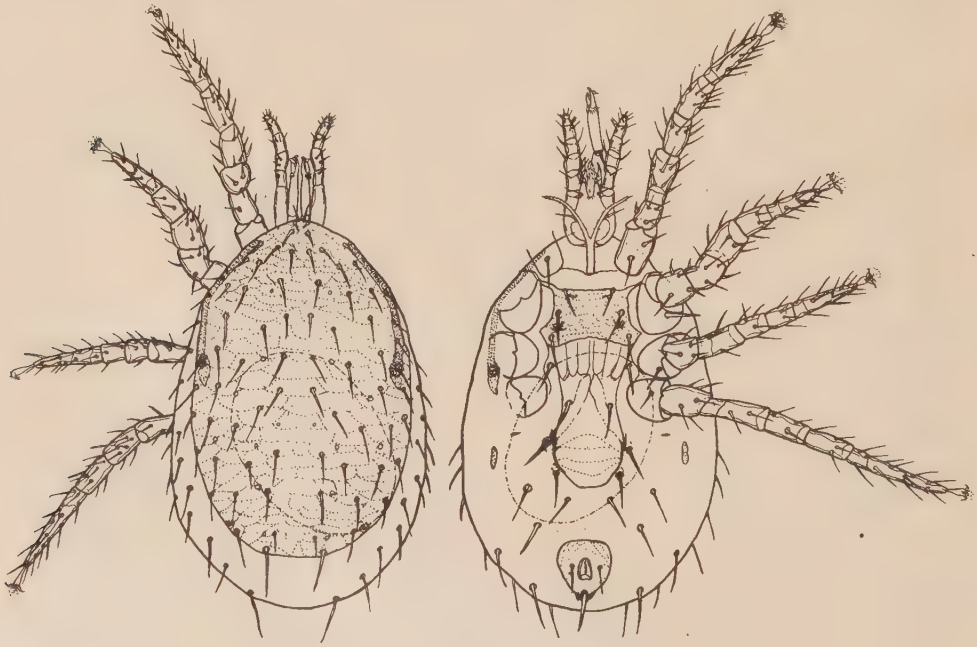

FIG. 4. Dorsal and ventral view of female.

It also projects a short distance caudad from the stigmal pore. The dorsal shield is entire and almost completely covers the dorsal side. There are at least 40 pairs of setae and numerous pores.

Female (Figs. 4 and 5).—Measurements are the average of 10 specimens. Length of body exclusive of mouthparts: 672 microns. Width of body: 472 microns. Sternal plate: length through center, 82 microns. Sternal plate: greatest width, between the projections opposite the 3rd setae, 169 microns. Genito-ventral plate: length, measured from posterior border of the sternal plate, 281 microns. Genito-ventral plate: greatest width, opposite 2nd pair of setae, 124 microns. Dorsal shield: length 608 microns; greatest width, 420 microns. Anal shield: length, 93 microns, width, 96 microns. Length of fore leg, 514 microns. Length of hind leg, 623 microns.

Mouthparts with 4 pairs of setae; one near the base, two (one long and one short) opposite the apex of the basal segment of the palp, and the fourth near the tip. The narrow ligula reaches slightly beyond the tip of the maxilla. The chelicerae, when fully extended, reach beyond the tip of the palp. Movable chela larger than the immovable, its tip incurved and with two blunt teeth

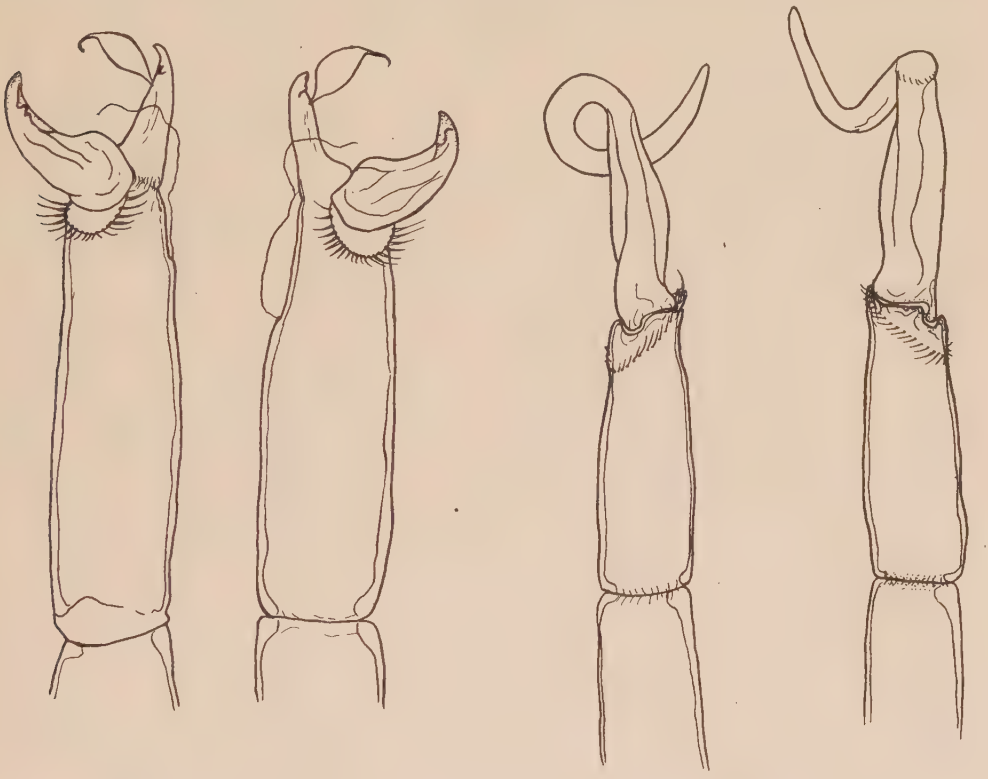

FIG. 5 (*left*). Chelicerae of female. FIG. 6 (*right*). Chelicerae of male.

below the tip; the immovable chela has three blunt teeth near the tip and the seta it bears is generally inflated with the tip curved in as illustrated, but some mounted specimens show the seta collapsed. At the base of the movable chela is a semicircle of close, fine setae. The sternal plate lies almost entirely between the second pair of coxae, with a narrow projection extending between the first and second coxae and a short, obtuse projection between the second and third. The anterior margin is nearly straight, the posterior margin is broadly concave. It bears the usual three pairs of setae; the first pair being on the anterior margin. There is a transverse pore below the first seta and an oblique pore below the second seta. The entire plate is marked with irregularly transverse lines. Only the outer margin of the endopodal plate is distinct. The genito-ventral plate has slightly convex sides, bears only one pair of setae, and falls far short of the anal plate. The anal plate is broadly triangular, with rounded margins. The paired setae are placed on a line running through the middle of the anus and are shorter than the unpaired, apical seta. There is a narrow, sclerotized line between the ventral plate and the second ventral seta, and a similar, horizontal line below the fourth coxa. The metapodal plate is small, longitudinal and irregularly elliptical. The fore coxa bears two long narrow setae, the second and third coxae each have a curved marginal seta and a straight submarginal seta, the fourth coxa

has a single seta. The peritreme reaches to the middle, or slightly beyond the middle of the first coxa. The dorsal shield is distinct, and is covered with a network of fine lines. The dorsal spines are quite heavy and long. Scattered among them are numerous pores. The legs have no unusually heavy or unusually long setae. The second pair of legs is the heaviest, the fourth the longest. In life, the first pair of legs serves a tactile rather than an ambulatory function.

Host: Sigmodon hispidus texianus (Audubon and Bachman).

Locality: Galveston County, near LaMarque, Texas.

Types: Cotypes (1 male, 3 females, 2 protonymphs, and 3 deutonymphs) in the U. S. National Museum, type no. 1532.

This form may be distinguished from other species of *Atricholaelaps* by the extremely long chela of the male and the longer and heavier body setae of the female.

My very best thanks are due Dr. Edward W. Baker for so kindly comparing specimens of this mite with those in the U. S. National Museum, and for other pertinent criticisms. Thanks are also due Miss Dorothy Eben for making the measurements given in the above descriptions.

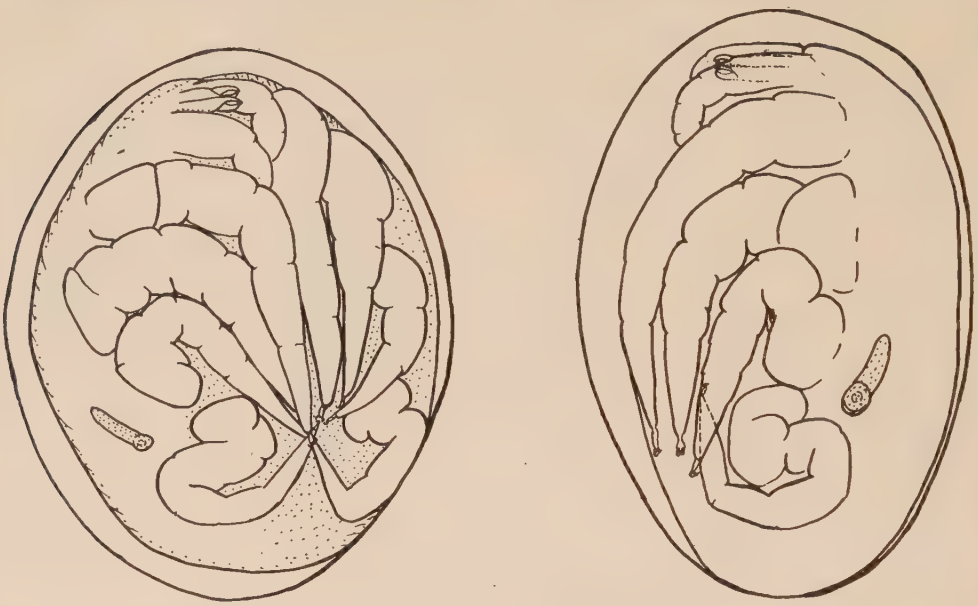

FIG. 7. Two views of the protonymph within the egg.

DISCUSSION OF *Atricholaelaps* EWING AND *Ischnolaelaps* FONSECA

Ewing (1929) erected the genus *Atricholaelaps* and characterized it as follows:

Body rather sparsely clothed with moderate setae. Chelicerae without brush of setae. Dorsal plate not sculptured. Sternal plate about as broad as long and broadest between second and third coxae. Genito-ventral plate falling far short of anal plate, anterior margin weak, less than four pairs of setae present. Anal plate subtriangular, not broader than long, with three setae. Metapodal plates minute or wanting. Neither the first or second legs in either sex calcarate, or with spines above. Coxae and most, or all, of the other leg segments without spines.

Type: Laelaps reithrodontis Ewing.

The new species described in the present paper does not meet all these characters but in the short discussion following the description of the genus *Atricholaelaps,* Ewing stated "... many species of *Laelaps* that are devoid of spinelike setae have less than four pairs of setae on the genito-ventral plates. They are placed in *Atricholaelaps.*" And the present species certainly meets those qualifications.

Fonseca (1935) erected the genus *Ischnolaelaps* and characterized it as follows:

Medium sized species, elliptical, poorly chitinized; genito-ventral plate slightly expanded posteriorly, showing only the genital pair of bristles; coxae without spines.

There is nothing in this brief description, nor in the descriptions of the three new species that follow it to distinguish this genus from *Atricholaelaps*. Radford (1939) described and illustrated six new species as *Ischnolaelaps* and none of these differ from Ewing's characterization of *Atricholaelaps*. Consequently, if those *Laelaptine* mites which have no coxal spines and only one pair of setae on the genito-ventral plate are recognized as a separate genus, it follows that Ewing's name (*Atricholaelaps*) is proper and Fonseca's (*Ischnolaelaps*) is therefore a synonym.

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THE USE OF DEHYDRATED, COAGULATED EGG YOLK IN THE PREPARATION OF A MEDIUM FOR CULTURING *ENDAMOEBA HISTOLYTICA*

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INTRODUCTION

Endamoeba histolytica has been successfully cultured both on media consisting of slants with liquid overlays and on entirely liquid media.

In our laboratory we have been testing the *in vitro* amoebicidal activity of chemicals on *Endamoeba histolytica* (University of Chicago strain) grown on E.L.E.S. medium (egg slant with overlay of 0.5% Lilly's liver extract) (Frye and Meleney, 1939) and also the National Institute of Health strain 103, grown on egg slants with modified Stone's Locke's overlay (Anderson, 1945). Due to the work involved in preparation of a medium consisting of a slant with an overlay, and the fact that the slants absorb a certain amount of the test chemical, we realized the need for an entirely liquid medium. After several attempts to culture the University of Chicago strain on St. John's (1932) heart broth medium without obtaining enough actively motile trophozoites for *in vitro* testing purposes, this medium was discarded. Balamuth and Sandza's (1944) medium, prepared with fresh egg yolks, has produced cultures containing numerous actively motile trophozoites in 48 hours.

Difco's Bacto-Coagulated Egg Yolk was selected as a replacement for fresh egg yolks. This product is, as the name implies, dehydrated, coagulated egg yolk, free from egg white, and seems to remain stable for several years when kept in a cool, dry place. The yolks of 500 or more eggs (Difco Co., 1945) are pooled in preparation of one lot of Bacto-Coagulated Egg Yolk, thus standardizing the product. A constant amount can be used in preparation of each lot of medium, tending to maintain reasonably uniform nutritional constituents.

In this study an attempt has been made: to determine the least amount of coagulated egg yolk that could be used in preparation of an entirely liquid medium, except for a small amount of solid rice powder; to produce a good growth of actively motile trophozoites in 48–72 hours; to simplify the medium preparation; to insure sterility by autoclaving the final tubed medium; and to obtain a more uniform standard of the constituents by pooling a large number of egg yolks.

PREPARATION OF MEDIA

Various amounts, i.e., 3 gm, 6 gm, 12 gm, 18 gm and 25 gm of coagulated egg yolk, representing 0.4%, 0.8%, 1.6%, 2.4% and 3.2%, respectively, were each allowed to stand 10 min. in 375 ml of 0.85% NaCl solution and then boiled 10 minutes. After cooling, the solutions were filtered through filter paper in a Büchner

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funnel and the original volume of 375 ml restored with distilled water. 375 ml of M/15 $Na_3PO_4 \cdot 12\ H_2O$ (pH 7.5) were added to each 375 ml of saline filtrate from the various amounts of coagulated egg yolk. After autoclaving 20 min. at 121° C, the pH of each of the five media was 7.4, as determined by a Beckman pH meter. The media were tubed in 5 ml amounts and autoclaved 20 min. at 121° C.

The Difco Bacto-Rice Powder was tubed in approximately 4 gm amounts, autoclaved at 121° C for 20 min. and dried 24 hr. at 58° C.

After the addition of approximately 40 mg of sterile rice powder, the media were incubated for 48 hr. at 37° C and examined grossly for contamination. The media containing *E. histolytica,* NIH (103), were streaked on sterile blood plates once a week to determine the presence of only "organism *t*."

PROCEDURE FOR TRANSFERS AND EXAMINATION

Two stock cultures of *E. histolytica* were used in this study. The University of Chicago strain, with unknown mixed bacterial flora, was previously cultured on E.L.E.S. (5 ml of egg slant, 5 ml of Lilly's liver extract overlay and 40 mg of rice powder). The NIH (103) strain, with bacterial flora "organism *t*," was previously cultured on Stone's Locke's egg slant (5 ml of egg slant, 5 ml of Stone's Locke's overlay and 40 mg of rice powder).

The original inoculation consisted of seeding six tubes of each of the five media with five drops of pooled 72 hr. culture of *E. histolytica,* University of Chicago strain and NIH (103), making a total of 30 tubes for each strain.

The cultures, after incubating 48–72 hr. at 37° C, were examined microscopically for *E. histolytica* by removing two drops of sediment with a sterile opsonic pipette. The growth and motility were recorded as follows (See Tables 1 and 2 and Fig. 1.):

TABLE 1.—*0.4% Difco Bacto-Coagulated Egg Yolk, University of Chicago strain of* Endamoeba histolytica

Transfers	Tube No.					
	1	2	3	4	5	6
Original June 14, 1945	+ A	+ P	+ A	+ A	++ A	++ F
First June 16	+ A	Few P	+ A	+ A	Few A	Few P
Second June 19	Very few P	Very few P	Few P	Very few P	Neg.	Neg.
Third June 21	Neg.	Neg.	Few A	Very few A	Neg.	Neg.
Fourth June 23	Neg.	Neg.	Few A	Very few P	Neg.	Neg.
Fifth June 26	Neg.	Neg.	Very few P	Very few P	Discarded	Discarded
Sixth June 28	Discarded	Discarded	Very few P	Few A	....	....
Seventh June 30	....	....	Neg.	Few A	....	....
Eighth July 3	....	....	Neg.	Neg.	....	....
Ninth July 5	....	....	Neg.	Neg.	....	....
Tenth July 7	....	....	Discarded	Neg.	....	....
Eleventh July 10	Discarded all tubes as no amoebae present					

Number of amoebae present:

Very few	1–2 amoebae per 18-mm coverslip
Few	3–15 " " " "
+	1–2 " " high power field
++	3–5 " " " " "
+++	5 " " " " "
++++	Over 5 amoebae per high power field

Motility:

P	Few actively motile trophozoites present
A	Half of trophozoites present are actively motile
F	Over half of the trophozoites present are actively motile
G	All actively motile trophozoites

In transferring cultures five drops of sediment were removed from each tube and inoculated directly into a fresh tube of medium. Incubated 48–72 hr. at 37° C.

DISCUSSION AND RESULTS

The two strains of *E. histolytica* used in this study were both laboratory strains. The University of Chicago strain has given ++A or better cultures on E.L.E.S. medium for use in *in vitro* amoebicidal testing. The NIH (103) strain with "organism *t*" averages +A cultures, now that we have succeeded in maintaining it for several months on Stone's Locke's egg medium.

After culturing the University of Chicago strain of *Endamoeba histolytica* for some time on Balamuth and Sandza's (1944) medium, using fresh egg yolk, we

TABLE 2.—*3.2% Difco Bacto-Coagulated Egg Yolk, University of Chicago strain of* Endamoeba histolytica

Transfers	Tube No.					
	1	2	3	4	5	6
Original June 14, 1945	+A	+++F	+A	++F	++A	++A
First June 16	++++F	++++F	++++F	++F	+++A	+++A
Second June 19	++++F	++A	++A	+++A	++A	++A
Third June 21	++++F	+++F	+++A	++++F	++A	+++A
Fourth June 23	++F	++++F	++A	+++F	++F	++F
Fifth June 26	++A	+++A	++A	++A	+A	+F
Sixth June 28	+++A	++A	++A	++++F	++A	++F
Seventh June 30	+++G	+++A	+++F	+++A	++A	+++F
Eighth July 3	++A	++F	++A	++F	+A	+A
Ninth July 5	++A	++A	+++F	++A	++A	++A
Tenth July 7	++++G	+++F	++++G	+++F	+++F	++++F
Eleventh July 10	+++A	++A	+A	+P	+P	+A
Twelfth July 12	++F	+P	++F	Few A	Few A	+A
Thirteenth July 14	++F	+++F	++++G	+++F	+++F	+++F
Fourteenth July 17	++F	++A	++A	++A	+A	++F
Fifteenth July 21	++++G	+++F	+++F	++A	++++F	++A
Sixteenth July 24	+++A	++A	++F	++A	++A	++F

became interested in determining if it could be prepared with dehydrated, coagulated egg yolks in order to simplify the preparation of the medium and to maintain reasonably uniform nutritional constituents.

Preparation of media for culturing *Endamoeba histolytica* which requires slants with overlays, is a rather laborious task. Each must be prepared separately and sterilized, then combined. The medium prepared with coagulated egg yolk is entirely liquid, except for a small amount of rice powder; when tubed and autoclaved, it is ready for use.

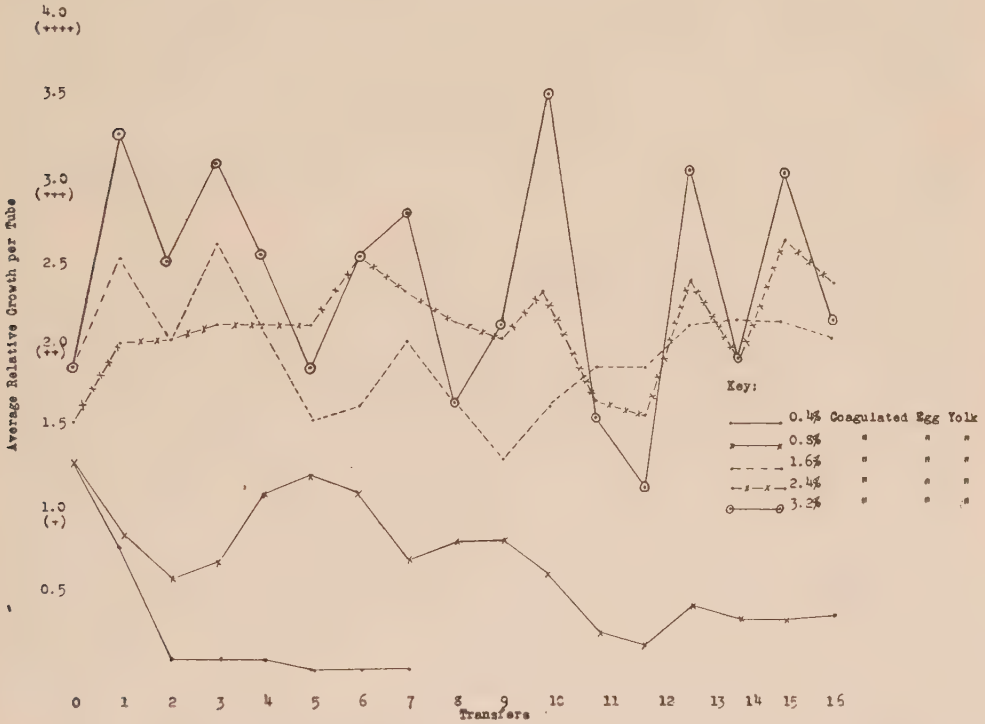

FIG. 1. In order to show the relationship of the average relative growth of *Endamoeba histolytica* (University of Chicago strain) in the six tubes of each of the five different lots of media, we prepared this graph plotting the average relative growth for the sixteen transfers. The total number of pluses (see Examination of Cultures), for the six tubes of each medium for each transfer, were totalled (+ considered as 1; ++, 2; +++, 3; ++++, 4; "Few," 0.5; and "Very Few," 0), and divided by six, giving the average relative growth for the six tubes for each transfer.

Apparently there is some substance or substances in fresh and dehydrated, coagulated egg yolk that is saline soluble, thermostable and a nutrient factor for *E. histolytica.*

The NIH (103) strain failed to grow in any of the media prepared with coagulated egg yolk. This failure may be due to lack of proper nutritional requirements or lack of ability of "organism *t*" to provide the necessary growth factors. These cultures were not contaminated and "organism *t*" was present as shown by streaking on sterile blood plates.

The University of Chicago strain began to show poor growth in the 0.4% medium after the first transfer and contained no amoebae in any tube after the seventh transfer (Table 1). The 0.8% medium maintains growth of amoebae, but

not in sufficient abundance for *in vitro* amoebicidal testing. The 1.6% medium contains the minimum amounts of coagulated egg yolk that can be used in preparation of a medium that produces cultures containing 3–5 trophozoites per high-power field, of which at least half are actively motile. The media containing 2.4% and 3.2% coagulated egg yolk gave more +++, ++++, F, and G cultures than did the media containing less coagulated egg yolk. (See Table 2 and Fig. 1.) The decrease in number of amoebae in the eleventh and twelfth transfers may be due to cyclic changes in the bacterial flora. This same phenomenon, due to unrecognized causes, has been noticed in our stock cultures of the University of Chicago strain on E.L.E.S. The same strain of *E. histolytica* was successfully carried through 16 transfers, made every 48–72 hr., in 1.6%, 2.4% and 3.2% Difco Bacto-Coagulated Egg Yolk media.

I (D.J.H.) should like to thank Parke, Davis and Company for the use of their equipment and materials, and Dr. O. Rulon of Wayne University for arranging for this study.

SUMMARY

1. *Endamoeba histolytica,* NIH (103) strain with "organism *t*" failed to grow in 0.4%, 0.8%, 1.6%, 2.4% and 3.2% coagulated egg yolk media.

2. *Endamoeba histolytica,* University of Chicago strain with unknown mixed bacterial flora, was carried through 16 transfers on 1.6%, 2.4% and 3.2% coagulated egg yolk media with good growth of motile amoebae.

3. 1.6 gm of coagulated egg yolk per 100 ml of solution was the minimum amount that gave ++A (3–5 trophozoites per high-power field and at least half of them actively motile) cultures of University of Chicago strain of *E. histolytica* in 48–72 hours.

4. Increasing the amount of coagulated egg yolk to 2.4% and 3.2% gave corresponding increases in the number of amoebae produced.

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FUNCTIONAL CYTOLOGY OF *ENTAMOEBA HISTOLYTICA*[1]

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Perusal of the literature reveals that very careful observations have not been made regarding the detailed cytology of *Entamoeba histolytica.* Few studies have been made to ascertain the functional relationships existing between various structures in the protoplasm. Observations to date have been concerned mainly with a) culture, b) life cycle, and c) nuclear sequence. Consequently, these observations have been under lower powers of the microscope, or with fixed and stained material (Dobell, 1928, and others). Hakansson et al (1936) made a study of the chromatoids and, although the report is not specific, the data indicate that only high dry magnification was used. Browne (1930), Kitabatake (1933) and Tsuchiya (1935) have studied *Entamoeba histolytica* using vital dyes. These authors have made no attempt to follow out the sequences involved in the functioning of the various structures that were stained. They appear to be mainly concerned with the tolerance of the amoebae for stains.

In recent years, considerable work has been done on the various rôles played by the different cytoplasmic constituents in cell metabolism (Mast & Doyle, 1935; MacLennan, 1941; Hopkins, 1938; Mast & Hopkins, 1941; Wilber, 1945; Mast & Bowen, 1945). However, these investigations have been made on free-living forms which are primarily aerobic. *Entamoeba histolytica,* while in the intestine, lives with little or no oxygen (Tappeiner, 1883; von Brand & Weise, 1932). Jacobs (1941) maintains that the usual redox potential of flourishing cultures of *Entamoeba histolytica* is about −0.33 V. Snyder and Meleney (1943) have maintained the organism in strictly anaerobic conditions. Therefore, this organism offers excellent opportunity for the study of an anaerobic form. Such a study should prove valuable both from the standpoint of fundamental cytology and in the diagnosis and prophylaxis of amoebic dysentery.

The need for an accurate description of the cytological constituents and their functional relationships in this vitally important amoeba led us to undertake the present work.

MATERIALS AND METHODS

Methods of culture.—In our investigation of the cytology of *Entamoeba histolytica,* we used a culture derived from Dobell's NRS strain which he originally obtained from monkeys. Before undertaking the study, we isolated a single individual and from this obtained a clone culture. We have cultured the amoebae according to Boeck and Drbohlav (1925) and Cleveland and Sanders (1930) and found these methods equally satisfactory. In preparing these media, however, we have used Ringer's solution to which a trace of sodium silicate was added (Hopkins, 1936). This silicate precipitates from solution traces of heavy metals that might be toxic, and results in a definite improvement in culture results.

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The bacterial flora of the cultures was maintained with a satisfactory uniformity by the usual bacteriological methods. The specific kinds of bacteria were unknown.

Methods of observation.—Microscopic observations were made with apochromatic objectives and compensating oculars using magnifications up to 1350 diameters. Both bright and dark field illuminations were used.

Very satisfactory definition of the more minute details was obtained using a drop of culture medium between a slide and a number one cover-glass. When it was desired to exclude excessive oxygen, the cover-glass was sealed with vaseline. Under these conditions, the amoebae would remain active and continue to feed for hours. Usually, however, a fresh drop of culture medium in which amoebae were actively flourishing, when placed under a 22 mm sq cover-glass would soon become highly reduced except around the edges as indicated by the bleaching of neutral red. According to Beutner (1933), neutral red is bleached at about −0.3 V. The amoebae live on the bottom, and it was found that neutral red was bleached in this region of flourishing cultures. When vaseline was not used, there was a certain amount of evaporation around the edges which increased the concentration of the salts. This concentration was less than one would normally expect since precipitation would occur at the edge of the cover-glass and partially seal the preparation. Observations made with unsealed preparations were always checked with sealed preparations or with fresh ones.

The majority of observations were made at room temperature, 24° (± 2°)C. Dobell (1928) maintains that lowering of the temperature below 37° C always results in abnormalities. We have, however, checked our observations made at room temperature with observations made at 37° C, other conditions remaining identical. The results were always the same except that all processes were speeded up at the higher temperature. For instance, amoebae treated with neutral red stained more readily at 37° than at 24° C, but the staining was strictly comparable. The neutral red was reduced sooner at 37° than at 24°. Locomotion was faster at the higher temperature, but we have not observed any abnormalities that could be attributed specifically to the lower temperature. Dobell kept cysts at room temperature and maintained that for at least three days no abnormalities resulted as a consequence.

RESULTS AND OBSERVATIONS

Feeding.—Greater difficulty was encountered in observing the feeding process in *Entamoeba histolytica* than was expected since it does not appear to feed in a highly oxidized medium. In fact, we have never observed feeding by an amoeba that was stained with an unbleached vital dye. We have, however, in a highly reduced medium observed this organism feeding upon rice starch grains, bacteria, fat globules and yeast. We have followed rice starch grains from the outside through ingestion, complete digestion and egestion of fecal matter.

The amoeba seems to have considerable power of selection of food since it will contact some bacteria, starch grains and various other objects without ingesting them, but will engulf others. When, however, a food particle is ingested, the advancing pseudopod passes over or close to the object without showing the slightest sign of adherence but as the body of the amoeba passes over the object, the tendency to adhere increases as the posterior end comes closer to the object. When the region which is just anterior to the posterior end reaches the food particle, the particle is

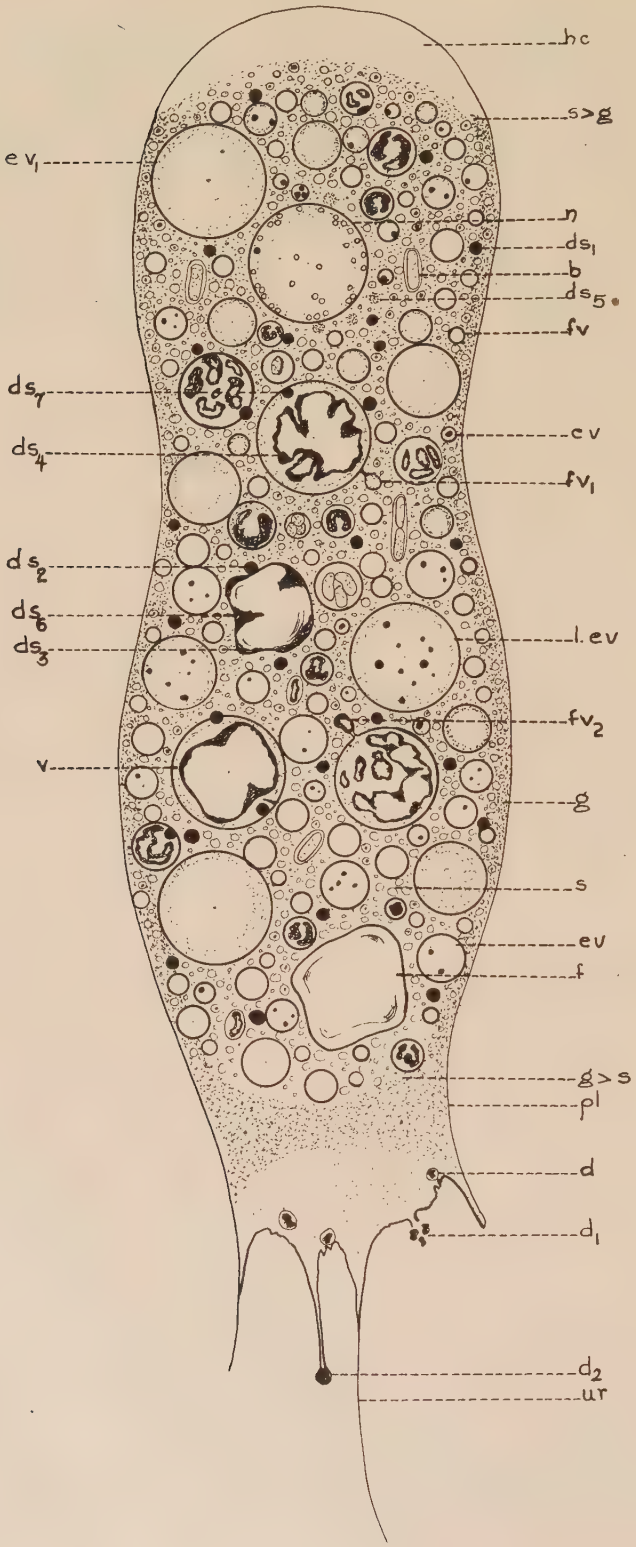
drawn into the amoeba just as a piece of glass coated with shellac is drawn into chloroform according to the classic experiment of Rhumbler (1898). No visible food cup is formed, and little or no fluid is included. Alexeieff (1933) has observed the ingestion of red blood corpuscles and finds that they are engulfed in a similar manner but much more rapidly. This would naturally be expected since his observations were made at 45° C.

The region of the cell where food particles are engulfed is also that region in which the plasmagel is becoming plasmasol most rapidly. When the particle is drawn into the protoplasm it is often impossible to distinguish it from the protoplasm itself. This is especially true of bacteria. Starch grains, however, can be followed by their markings. It is only a matter of a few seconds before an engulfed food particle can be observed to move forward in the plasmasol stream.

Digestion.—Soon after the food particle gains entrance into the cytoplasm, small, viscous, spherical bodies can be seen to attach to its surface. These bodies may be observed to flatten themselves out on the surface of the food (Fig. 1, $ds_{2,3}$).

These spherical bodies appear throughout the cytoplasm and are structures usually identified as granules although their fluidity does not justify this terminology (Fig. 1, ds_1). Attempts to find their origin revealed the fact that they are formed near the nucleus. On occasions, in a quiescent amoeba which was flattened out under the cover-glass, we found food vacuoles containing starch grains lying near the nucleus. If, in such an amoeba, the small strip of protoplasm between the vacuoles and the nucleus were observed closely, small spherules were seen to form *de novo* in the cytoplasm contiguous with the nucleus (Fig. 1, ds_5). They then entered the food vacuole and spread out on the surface of the starch grain. On other occasions the nucleus in an amoeba under pressure was seen lying so that the outer edge was resting in the hyaline cytoplasm next to the plasmalemma. Since the cytoplasm in this area was clear, one could see definitely that these spherules did not wander in from some other region but arose *de novo* from the optically clear cytoplasm. Nothing could be observed visibly coming from the nucleus into the cytoplasm. It would seem as if a substance were diffusing from the nucleus, reacting with the clear cytoplasm and the products of this reaction coalescing to form visible globules, the viscous, spherical bodies. This is fairly conclusive evidence that the nucleus plays an important rôle in the formation of these spherules.

After they have attached themselves to the surface of the food particles, these spherules have a corroding effect on the food (Fig. 1, ds_6). A starch grain which has been in the amoeba for some time becomes literally coated with these flattened spherules. Some of these spherules may break loose into the cytoplasm as vacuoles (Fig. 1, fv_1). When an abundance of these spherules is flattened out on a starch grain, and their boundaries come in contact, they may coalesce. This coalescence may continue to such an extent that the starch grain is surrounded completely by the coalesced spherules. It is in this way that the vacuole (Fig. 1, v), which can often be observed around a starch grain during the process of digestion, arises. The clear fluid between the starch grain and membrane results from the osmotic activity of the soluble products of digestion which draw water from the cytoplasm. Even after this vacuole has been formed around the starch grain, we have observed that the small spherules continue to reach the starch grain and flatten out on it. In so doing, they apparently pass without any difficulty through the vacuolar membrane.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When they are flattened out on the grain, they remain separate and distinct from the vacuolar fluid (Fig. 1, $ds_{4,7}$). It is thus obvious that the viscous, spherical bodies are digestive in function and can be designated as *digestive spherules.*

The digestion due to the action of the spherules continues the breakdown of the starch grain. Fragments of the grain have been seen, frequently, to break loose from the main mass and pass directly out of the vacuole into the cytoplasm (Fig. 1, fv_2). Spherules without solid pieces also separate from the surface of the starch grain and pass out into the cytoplasm (Fig. 1, fv_1), becoming small, clear, cytoplasmic vacuoles (Fig. 1, fv). In passing out from the large food vacuole, these spherules suffer no more impedance at the membrane than when they originally entered.

Finally, the main mass of the starch grain is so digested that it fragments; the pieces pass singly or in groups into separate vacuoles. Here digestion is continued by the spherules until only the indigestible wastes remain. The resulting fecal vacuoles (Fig. 1, d) then are relegated to the posterior end for elimination (Fig. 1, d, d_1).

Thus we see from the above description how different kinds of food vacuoles are derived. The addition of distilled water to the outside medium will cause swelling of a) large food vacuoles containing starch grains, if the latter have been in the cytoplasm long enough for digestion to begin, b) small, clear food vacuoles and c) sometimes of small vacuoles containing food residues. The small vacuoles with residues do not always swell. This is especially true if they have become isolated in the posterior end of the amoeba. It should be emphasized that food vacuoles that have just formed by ingestion will not swell upon the addition of distilled water.

These facts are explained by the conclusion that the food vacuoles have an appreciable osmotic activity only after digestion of food has occurred. It is interesting to note that the fecal vacuoles located in the posterior end have, for the most part, lost their osmotic activity.

Nutrition and food storage.—It is impossible to trace, microscopically, the fate of the smallest clear vacuoles containing digested food, but the following observations would seem to indicate the sequence in which food is utilized.

The cytoplasm of amoebae moving and dividing actively (Fig. 1) is very fluid. It contains numerous very small refractile bodies or vacuoles, 0.1 – 1.0 micron in diameter; also vacuoles of intermediate size, each containing one or two small granules (Fig. 1, ev). Large clear vacuoles are scarce. The larger vacuoles present contain undigested food (Fig. 1, v) or granules (Fig. 1, l.ev). The abundance of refractile bodies and of granules in vacuoles is even more evident in amoebae which have been exposed to the oxygen of the air recently. In other words, the cytoplasm appears exceptionally granular. On the other hand, actively reproducing amoebae

FIG. 1. Diagram showing detailed structure of an active trophozoite of *Entamoeba histolytica.* d, fecal vacuole; d_1, fecal vacuole exploding to outside; d_2, fecal vacuole in uroid; ds_1, digestive spherule; ds_2, digestive spherule attaching to food; ds_3, digestive spherule flattening out on food; ds_4, digestive spherule on starch grain distinct from vacuolar fluid; ds_5, digestive spherule arising *de novo* around the region of the nucleus; ds_6, corroding effect of digestive spherules; ds_7, digestive spherule in food vacuole distinct from vacuolar fluid; ev, excretory vacuole; ev_1, clear excretory vacuole; f, food particle; fv, clear food vacuole; fv_1, digestive spherule breaking loose into cytoplasm as a clear food vacuole; fv_2, fragment of food passing into cytoplasm; g, plasmagel; g>s, region of gel-sol transformation; hc, hyaline cap; l.ev, large excretory vacuole; n, nucleus; pl, plasmalemma; s, plasmasol; s>g, region of sol-gel transformation; ur, uroid.

from a flourishing culture which have been sealed under a cover-glass with vaseline without exposure to air, show this condition for a time only. As the supply of oxygen decreases due to its utilization by the bacteria and amoebae, larger and larger clear vacuoles appear until the amoebae become literally packed with them (Fig. 2, A). In such amoebae, flattened considerably by a cover-glass, these vacuoles can be seen to arise by coalescence of very small vacuoles. Small vacuoles can be observed to arise *de novo* in clear cytoplasm.

The addition of the reducing agent, sodium hydrosulphite, in very small quantities to a preparation containing active amoebae will cause the formation and accumu-

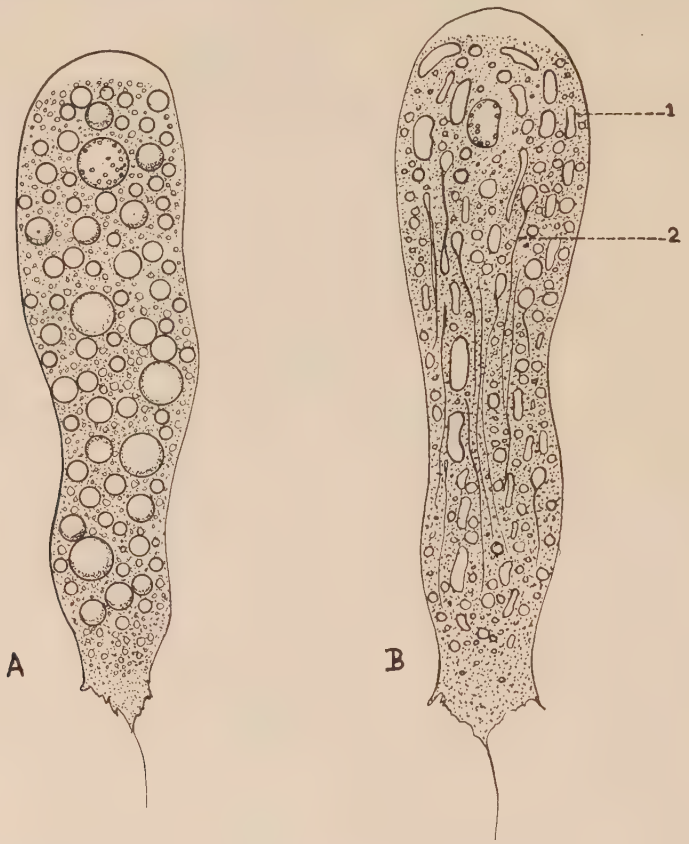

FIG. 2. A. Amoeba packed with clear vacuoles due to decrease in oxygen supply. B. Amoeba in pre-chromatoidal stage, showing viscous, distorted vacuoles (1) some of which have been torn in two leaving long tailpieces (2).

lation of larger clear vacuoles. The addition of excessive amounts of the reducing agent stops all activities and the amoeba dies. Conversely, the addition of small quantities of hydrogen peroxide to preparations containing amoebae, packed with clear vacuoles, causes immediate fragmentation of the larger clear vacuoles into small ones and the appearance of granules in many of them. The addition of an excessive amount of hydrogen peroxide kills the amoeba.

It has been frequently observed in the literature (and we have confirmed the fact) that amoebae degenerate in shallow cultures or in cultures in which bacterial activity is low. Amoebae on slides left exposed to the air show this same condition.

This degeneration is not encystment, since successful subcultures cannot be obtained from such amoebae. These degenerates are characteristically granular and lack vacuoles. The more degenerate, the fewer the vacuoles present.

The amoeba is thus seen to be sensitive to oxygen. It does not seem to be able to control its oxidative processes. The degree of oxidation of the foodstuffs and even protoplasm is directly related to the oxygen tension. The optimum oxygen tension for growth, reproduction and locomotion, as previously stated, appears to be well below that which reduces neutral red to its leuco base (about −0.4 V). Amoebae that stain with neutral red show few clear vacuoles but numerous vacuoles containing granules. When reduction has occurred and the neutral red in the amoebae is reduced, the clear vacuoles become more abundant. Thus a redox potential above the optimum causes destructive oxidation while below that potential normal oxidation of foodstuffs is prevented. Amoebae in an abundance of oxygen stop feeding, consequently there is a complete depletion of foodstuffs; but amoebae in a reduced medium, perhaps even in a medium too reduced for normal oxidation, continue to feed, and unoxidized food accumulates in vacuoles in the cytoplasm. It is thus that the formation of glycogen masses and chromatoidal bodies is brought about.

The synthesis of protoplasm from the material in the clear food vacuoles is probably dependent upon four factors: a) oxygen, b) oxidative enzymes, c) available food and d) synthetic enzymes in the cytoplasm. Assuming that the right conditions are present, a certain amount of oxidation takes place, and the resulting energy is used in the transformation of foodstuffs into protoplasm.

A logical conception of the ultimate fate of the clear food vacuoles is that oxidation which naturally would occur in the peripheral regions of the vacuole first would cause a change in permeability of its membrane, thus allowing foodstuffs to diffuse into the cytoplasm where they would be acted upon by ever-present synthetic enzymes to bring about the synthesis of cytoplasmic substances. When oxidation of the contents of the food vacuole and the diffusion of other foodstuffs has been completed, there remains in the vacuole only insoluble or indiffusible residues, the granules observed above, which are the excretory granules (Taylor, 1923, and others). This process of complete utilization of the food vacuoles is prevented if there is a deficiency of oxygen, in which case, the small vacuoles coalesce to form either larger and larger vacuoles or chromatoids.

We have followed the formation and development of the chromatoids and we find that they are formed by the coalescence of the clear vacuoles. As the degree of reduction increases, these vacuoles become more viscous and plastic (Fig. 2, B). When they are forced through narrow channels, their shape is distorted and even when the pressure is removed they remain distorted (Fig. 2, B, 1). Sometimes, a large vacuole may be torn in two, leaving long fibrous tailpieces (Fig. 2, B, 2). Small vacuoles may be pressed into fibers and remain. Thus, the flowing cytoplasm at times appears to be fibrous. This seems to be reminiscent of the filamentous structures observed by Kofoid and Swezy (1925) in the cytoplasm of *Entamoeba gingivalis*.

During the precystic stage and cyst formation, these plastic bodies become molded into rods, spherules and other shapes—the chromatoids, so-called because of their affinity for nuclear stains. This staining property can be accounted for by the fact

that the chromatoids, as observed above, have been formed from the clear food vacuoles which arise during the digestion of food by the digestive spherules which, as we have seen, originate from the nucleus.

The so-called glycogen bodies in *Entamoeba histolytica* are not bodies but regions where glycogen has accumulated, as evidenced by their ability to stain with iodine. In the precystic amoeba, the glycogen appears to be concentrated in the posterior end. The glycogen disappears from the cyst before the chromatoids.

The chromatoids are present in the earlier stages of the cyst but have generally disappeared by the four-nucleate stage (Dobell, 1928). Hakansson et al (1936) maintain that the chromatoidal substance is not used up but merely that the chromatoids disintegrate into what he calls "latent chromatoidal material," and that they reform when the cysts are placed in water or in other liquid media. Dobell (1928) believes that the chromatoids are composed of stored food which is utilized during encystment. Our observations support this viewpoint. The breakdown of the chromatoids during encystment is obviously the same process and caused by the same agency as the breakdown of large food vacuoles during active metabolism, but much slower. The slow metabolic rate during encystment is the result of the relatively low permeability of the cyst wall to oxygen, water and other substances. It is natural to assume that the water content of the precystic amoeba and the cyst is lower than that of the active trophozoite since the viscosity of the chromatoids is probably a result of polymerization of the foodstuffs, and the formation of glycogen is certainly a polymerization.

Excretion and egestion.—We have observed a) fecal wastes, the indigestible residues of engulfed food, b) vacuoles containing small refractile bodies which we have called excretory granules, c) vacuoles containing only clear fluid or semifluid material and d) undigested contents of food vacuoles being eliminated from the amoeba.

As a general rule, undigested food is relegated to the posterior end for elimination. This is accomplished in an interesting manner. The vacuoles containing the waste become located in the posterior end which is well behind the region in which the most active gel-sol transformation is taking place. At the point where the gel-sol transformation is taking place (Fig. 1, g>s), there is a distinct contraction of the outer protoplasm which tends to squeeze in both directions, anteriorly and posteriorly. It is this posterior vector of the squeezing force which forces the fecal vacuoles out into posteriorly projecting processes, the uroids (Fig. 1, ur). Sometimes this force is sufficient to cause the fecal vacuole to explode outside, releasing its contents (Fig. 1, d_1). At other times, however, the fecal vacuole will remain in the uroid and, since the uroid is sticky, it adheres to the substratum or to nearby objects so firmly that it can be drawn out into a very long slender filament. This breaks, leaving behind the end of the uroid containing the fecal vacuole (Fig. 1, d_2).

The excretory vacuoles (Fig. 1, l.ev) usually contain a few or many granules. These vacuoles, when they come to lie near the surface of the amoeba, seem to have the power of causing the breakdown of the plasmagel which in turn causes the formation of a pseudopod at that point. The rush of the cytoplasm into the pseudopod carries the vacuole into its tip. When the pseudopod is retracted, the vacuole is trapped. The retracting pseudopod is similar in all respects to the posterior end of

the amoeba. The force of contraction results in two vectors of force: one directed towards the amoeba and the other towards the tip of the retracting pseudopod, forcing the vacuole up against the membrane of the pseudopod, causing its rupture to the outside. The membrane of such vacuoles will adhere to the outside membrane if it contacts it for only an instant. One of these vacuoles was observed to touch the outer membrane and immediately the flow of protoplasm became directed so as to sweep it away. In tearing loose from the outer membrane, the contacting surfaces were drawn out into a strand which broke with a snap.

Occasionally, clear vacuoles have been seen to contact the outer membrane and empty to the outside (Fig. 1, ev_1). These clear vacuoles remind one of contractile vacuoles in appearance and in manner of elimination of their contents. They sometimes empty to the outside with violence, but at other times their contents seem to be more viscous, in which case the voidance is slower. It is interesting to note that excretory vacuoles, whether clear or containing granules, are not eliminated from any particular region of the cell.

When an amoeba is going into the precystic stage, it eliminates all waste and the residues of undigested food in the food vacuoles. The latter is probably due to the fact that the cytoplasm has reached the saturation level. Such undigested food may be eliminated in the same manner as fecal vacuoles or, at times, in the manner of the excretory granules.

From these observations, it can be seen that the extreme posterior end of this amoeba cannot be clearly defined as nephridioplasm as can that of *Amoeba lacerata* as observed by the author (unpubl.).

Osmotic activity of cytoplasmic inclusions.—As stated previously, the food vacuoles have osmotic activity in proportion to their digested food content. The excretory vacuoles are variable in reference to their swelling when distilled water is added to the outside medium. They will all swell to a certain extent regardless of whether they contain numerous or few granules. Since they are probably derived from food vacuoles whose contents have been oxidized, it is possible that their osmotic activity is due in some extent to unoxidized food and also partly to soluble products of oxidation.

The osmotic activity of the cytoplasmic constituents can be compared with that of the external medium by crushing the amoeba, exposing the vacuoles and nucleus to the medium and freeing them from the amoeba whose tensile strength may prevent their absorption of water. We have done this by placing the amoeba under a cover-glass in a very thin layer of culture medium; then, by holding a needle just above the amoeba and exerting pressure gradually. This easily ruptures the amoeba, and has been done, observing continuously the operation and the results under the microscope. The results obtained may be summarized in a single statement: all vacuoles and the nucleus when exposed to the outside medium swell, but not extensively. This would indicate that the osmotic activities of the nucleus and vacuoles are higher than that of the outside medium. It is very probable, however, that the observed swelling is largely due to the release of the vacuoles and nucleus from the restraining elastic strength of the plasmagel and the plasmalemma.

Locomotion.—The mechanism of locomotion of *Entamoeba histolytica* is very similar to that described by Mast (1926) for *Amoeba proteus*. The cell consists of a distinct plasmalemma (Fig. 1, pl), plasmagel (Fig. 1, g) and plasmasol (Fig. 1, s).

There is, as in *Amoeba proteus,* a cycle of changes of the protoplasm from gel to sol posteriorly and of sol to gel anteriorly (Fig. 1, g>s, s>g). Posteriorly, the inner plasmagel breaks down into plasmasol; this breakdown is associated with the contraction of the outer layers of gel which forces the recently formed sol forward. The sol thus forced forward pushes through an anterior weakening in the gel forming a pseudopod or hyaline cap (Fig. 1, hc). The anterior weakening in the gel is not always a complete breakdown of the gel, but does result in its increased porosity; this allows clear hyaline protoplasm to flow through the gel and against the plasmalemma which, being elastic, is stretched in the direction of the flow thus forming a typical hyaline cap at the anterior end of the advancing amoeba (Fig. 3, A, a, B, b, C, c). This hyaline cytoplasm in the cap gelates within a few seconds. The next break-through of the hyaline cytoplasm comes between this and the plasmalemma (Fig. 3, B, a–b, C, b–c). In *Entamoeba histolytica,* this break-through of the clear

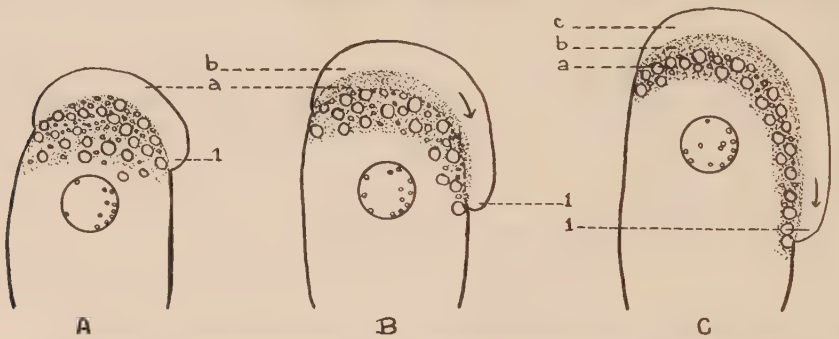

FIG. 3. Diagram of pseudopod formation in *Entamoeba histolytica.* Hyaline cap (a) gelates and hyaline fluid breaks through (a) and forms hyaline cap (b) which gelates and more hyaline fluid breaks through (b) to form hyaline cap (c). Note the plasmalemma tearing loose at (1).

cytoplasm occurs in spurts, thus "eruptive" pseudopod formation, a feature by which this organism is often recognized. The escape of this hyaline cytoplasm through the gel is often extensive; the membrane is not only pushed forward but the hyaline cytoplasm may flow posteriorly from the tip ripping the plasmalemma loose from the plasmagel (Fig. 3, A, 1, B, 1, C, 1), stretching it laterally and extending the hyaline cap sometimes as far back as the posterior end. This last observation shows that there is no hyaline fluid layer between the plasmalemma and the plasmagel as has been described by Mast (1926) for *Amoeba proteus.* The membrane, unless torn loose in the manner just described, definitely adheres to the plasmagel. The plasmalemma is differentiated from the gel in that it is tougher and more elactic. This being true, it is impossible for it to slide over the gel as is maintained by Mast to be the case in *Amoeba proteus.*

The factors that determine pseudopod formation would seem to be any agents which will cause a localized weakening of the gel layer. The nucleus of *Entamoeba histolytica* is usually located in the anterior region of the advancing pseudopod just behind the hyaline cap. There are, however, frequent exceptions to this rule, in which the nucleus is not near the point at which the pseudopod is forming. As indicated in another connection, we have observed certain large vacuoles which appear to influence pseudopod formation. Such vacuoles have been followed extensively. When they come to lie against a given spot in the plasmagel layer for a time, the gel

at this point will dissolve completely and consequently a pseudopod will be formed, forcing the vacuole out into its tip. The same vacuole may be observed to cause the repeated formation of pseudopods. Such a vacuole will always cause the formation of a pseudopod if it lies for a time in the plasmagel, thus clearly indicating that something is coming from the vacuole which is causing the solation of the gel. These vacuoles are eventually trapped in a pseudopod and their contents expelled to the outside. A striking difference between these vacuoles and their relation to pseudopod formation and the nucleus and its relation to pseudopod formation is that the vacuoles go up to the plasmalemma but the nucleus rarely does. Just anterior to the point where the nucleus comes to rest, there is a gel layer. Therefore, we find ourselves inclined to agree with Kofoid and Swezy (1925) that the nucleus does not play an obvious rôle in the initiation of pseudopod formation.

DISCUSSION

The metabolic cycle.—The foregoing evidence justifies the following outline of the metabolic cycle in *Entamoeba histolytica*. The accompanying diagram, Fig. 4,

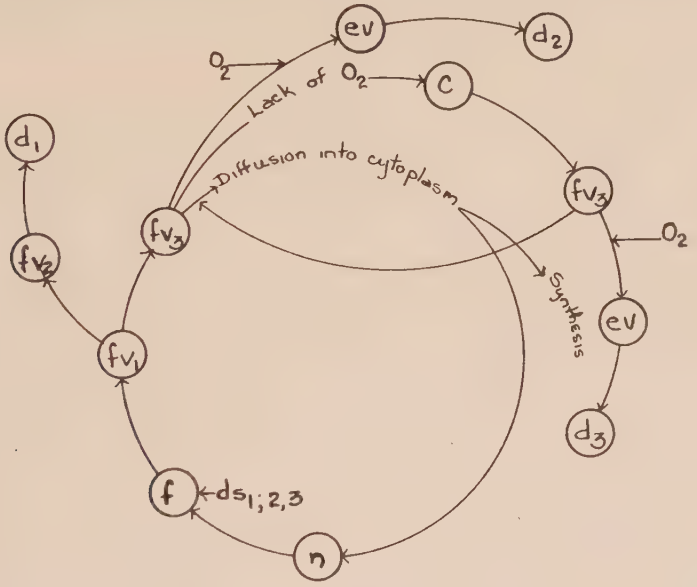

FIG. 4. The metabolic cycle in *Entamoeba histolytica*. See Fig. 1 and text for legend.

gives the complete cycle. We will discuss only those parts which need clarification.

From our observations, it appears fairly certain that substances diffusing from the nucleus (n) cause the formation of the digestive spherules (ds_1) which become dispersed in the cytoplasm and are abundant throughout. The ground substance of these bodies may be of a lecithin-like nature. They are definitely attracted to the food vacuoles and adhere (ds_2) and flatten out (ds_3) on the food particle (f); they apparently carry the digestive enzyme. The evidence for this is that corrosion of the food particles occurs whenever they are present, but not when they are absent. Some coalesce with one another while others remain distinct. While on the surface of the food particle, they also absorb digested food. From this point on, these spherules have all the properties ascribed to food vacuoles. When a concentration

approximately equivalent to that of the cytoplasm is attained, the bodies (fv_1) leave the food (f) and pass into the cytoplasm sometimes carrying with them, along with the digested food, a piece of undigested food (fv_2). In the cytoplasm, they fragment repeatedly (fv_3) until they are of extremely small size, perhaps submicroscopic. Those vacuoles receiving indigestible residues are eliminated (d_1). It is probably in a subdivided condition that digested food is either oxidized or removed and incorporated into the cytoplasm. If there is a deficiency of oxygen, the vacuoles may coalesce repeatedly to form chromatoids (c), storage bodies. In any case, when oxygen is plentiful, oxidation of these foodstuffs results in the liberation of energy, disintegration of foodstuffs, and apparently the formation of excretory vacuoles (ev) which retain the waste by-products sometimes in solution but usually in the form of granules. The chromatoids in the cyst are fragmented before extensive oxidation occurs. The excretory vacuoles formed in the cyst are retained until excystation, while in the trophozoite these vacuoles are expelled soon after they are formed (d_2, d_3).

The oxidation of the foodstuff releases energy which is used in the vital activities of the cell such as locomotion, the synthesis of cytoplasm, and its reduction. The nucleus probably absorbs reduced substances from the cytoplasm which are utilized in its syntheses.

Causey (1925) in *Entamoeba gingivalis* described the adherence of bodies (which he identified as mitochondria) to food vacuoles. Horning (1926, 1927, 1928) observed structures identified as mitochondria arising in the region of the nucleus of *Paramecium, Nyctotherus* and *heterotrichs* entering the food vacuoles and adhering to the food particles. He claimed that they caused the food to dissolve and thus were enzymatic in action. These so-called mitochondria of Causey and Horning, especially those of the latter, correspond in their major details with our digestive spherules. In the marine amoeba, *Flabellula mira,* Hopkins (1938) observed neutral red-stained vacuoles attaching and spreading out on engulfed bacteria. These are undoubtedly comparable also to the digestive spherules. Wenrich (1941) has observed, in fixed and stained preparations of *Entamoeba muris* and *E. ranarum,* "digestive granules" (spherules) stained with iron haematoxylin attached to engulfed starch granules.

Mast and Doyle (1935) in *Amoeba proteus* and Wilber (1945) in *Pelomyxa carolinensis* have observed neutral red-stained bodies coming from the food vacuoles and going into the cytoplasm. They speak of them as "vacuole-refractive bodies." In addition, Wilber has observed crystals coming from the food vacuoles and entering the cytoplasm. These observations are paralleled by ours on the behavior of the clear food vacuoles. Mast and Doyle, in addition, maintain that "beta granules" function in transferring digested food substances from food vacuoles and crystal vacuoles to the "refractive bodies" (storage bodies). Wilber (1945) was unable to confirm this in *Pelomyxa.* The "beta granules" of Mast and Doyle (1935) and Wilber (1945) probably are analogous to the excretory granules in *Entamoeba histolytica.* The "condensation granules" of *Flabellula mira* (Hopkins, 1938) are also analogous to excretory granules. In the fresh-water amoeba, *Amoeba lacerata,* the writer has found that the excretory substances are in solution and are eliminated by the contractile vacuole. As we have noted, this may also occur in *Entamoeba histolytica.*

It is interesting to note that *Entamoeba histolytica,* in reference to excretion, stands intermediate between *Flabellula mira,* a marine amoeba, and *Amoeba lacerata,* a fresh-water form, as is natural to expect since the concentration of its normal medium is intermediate between these other two. *F. mira* excretes excretory granules; *E. histolytica* excretes granules and dissolved waste; and *A. lacerata* excretes only dissolved wastes.

The "refractive bodies" of *Amoeba proteus* and *Pelomyxa carolinensis* are storage bodies and apparently analogous to the chromatoids.

Vital dyes.—Authors concerned with the functional cytology of the Protozoa have made considerable use of vital dyes. It was impossible for us to make continuous observations of granules or structures by means of any vital dye. Especially was this true when amoebae were in media sufficiently reduced to allow for normal activities since the vital dyes available to us were all reduced to their leuco form at a higher redox potential. We consider this inability to use vital dyes not so much of a handicap as it would seem at first, since we have been able to follow the course of the digestive spherules without stain while previous attempts by other workers have failed to do so with stained material. This failure could be due to the fact that stains have a tendency to inhibit normal functioning.

SUMMARY

1. *Entamoeba histolytica* thrives best at a redox potential below that which is required to reduce neutral red to its colorless form (about −0.4 V). Greater reduction than this is associated with encystment while greater oxidation is injurious and is associated with degeneration. The rate of oxidation of foodstuffs appears to be controlled by the oxygen tension of the outside medium rather than by the amoeba itself.

2. Food is engulfed in the region just anterior to the posterior end where sol-gel transformation occurs most rapidly. It is taken in with little or none of the external medium.

3. Digestion is brought about by enzymes carried to the food vacuole by *digestive spherules* which arise at the periphery of the nucleus, obviously due to the action of the substances diffusing from the nucleus into the cytoplasm.

4. The substance of the spherical bodies which cause digestion absorbs the digested food and carries it from the food vacuole into the cytoplasm in the form of clear vacuoles which fragment into extremely small size, and there, foodstuffs may be oxidized, or synthesized into protoplasmic compounds, depending on the oxidative rate.

5. In the absence of sufficient oxygen, these clear vacuoles or fragments coalesce to form storage bodies, the chromatoids.

6. Oxidation results in the formation of excretory vacuoles containing soluble waste or excretory granules or both.

7. Excretion is accomplished by the elimination of these excretory vacuoles at almost any point on the surface.

8. Egestion of fecal waste occurs from the posterior end by way of the posterior projecting processes, the uroids, which are torn loose from the body of the cell. Egestion may also occur by rupture of vacuoles to the outside.

9. The mechanism of locomotion in *Entamoeba histolytica* is essentially the

same as in *Amoeba proteus* as described by Mast, except that pseudopod formation is eruptive and there is no hyaline fluid layer in the posterior regions of the cell between the plasmalemma and the plasmagel.

10. All vacuoles and the nucleus are osmotically active, having osmotic concentrations perhaps only slightly higher than those of the outside medium.

11. Certain vacuoles may cause pseudopod formation by the diffusion of substances from the vacuoles which solate the plasmagel. The nucleus does not act in this manner.

12. It is suggested that the use of vital dyes prevents the normal digestion, absorption and assimilation of food.

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CHEMICAL STUDIES ON EGG-WHITE MEDIUM FOR THE CULTIVATION OF *ENDAMOEBA HISTOLYTICA*

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In a previous paper on the diphasic medium employed for the cultivation of *Endamoeba histolytica,* von Brand, Rees, Jacobs, and Reardon (1943) reported their investigations on some changes occurring between base and overlay with the passage of time. Diffusion of reducing substances occurred over a period of a week in media in sterile test tubes stored at 37° C. Diffusion was more rapid in media in Erlenmeyer flasks because of greater relative surface of the base. As expected, the addition of glucose to the overlay in amounts up to 300 milligrams per cent retarded diffusion. In cultures of *E. histolytica* with a single species of bacteria designated as organism *t* and of organism *t* alone the reducing substances were almost completely utilized after incubation for 24 hours. These data do not indicate direct utilization of reducing substances by *E. histolytica* since it could not be grown without bacteria. Although the amoeba ingests starch which may function in its carbohydrate nutrition, in our cultures it must also depend indirectly on soluble carbohydrate because of its dependence on the growth of organism *t*. The work was continued in the present studies which in addition include diffusion experiments on fats and nitrogenous compounds. The amoebae probably require the latter two kinds of nutriment and it is therefore important to measure the amounts diffusing into the overlay. In addition some studies were conducted on spontaneous oxidation of medium with the objectives of finding chemical explanations for cultural data showing decreased yields of amoebae in medium that was stored for two weeks or longer in contact with air (Rees and Reardon, 1945).

Chemical changes that might possibly account for observed variations in yields of amoebae from media prepared under slightly different conditions were also investigated. The shape of the container, the relative amounts of base and overlay, and the method of preparing the coagulum were factors affecting yields of amoebae—von Brand, Rees, Jacobs, and Reardon (1943), Rees, Bozicevich, Reardon, and Jones (1942), Rees, Bozicevich, Reardon, and Daft (1944), and Rees and Reardon (1945). The present investigation furnishes a basis on which the changes resulting from the above factors can be approached experimentally.

Since publication of the work on reducing substances (von Brand, Rees, Jacobs, and Reardon, 1943) some progress has been made in identifying vitamins and cholesterol as growth factors for *E. histolytica*—Snyder and Meleney (1943), Rees, Bozicevich, Reardon, and Daft (1944). In our work the previously used whole-egg medium was replaced with egg-white medium to which the above factors were added.

In the present work a standard emulsion prepared from three parts of egg white

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to one part of Locke's solution was used. In designated cases this emulsion was analyzed before coagulation; in other cases it was analyzed after the emulsion was inspissated and sterilized in Florence flasks of 250 ml capacity in amounts of 25 ml or 50 ml per flask. This base was studied without overlay and also with 100 ml or 200 ml of an overlay of Locke's solution. With exceptions as noted, powdered egg shell in the amount of 3 gm per flask was mixed with the emulsion before inspissation. In our cultural studies (Rees, Bozicevich, Reardon, and Jones, 1942) egg shell is regularly used in this manner as a buffering agent. The flasks were closed with sterile rubber stoppers to prevent evaporation, and in studies on oxidation they were also provided with a mercury seal. The flasks were stored for intervals either at 10° C or 37° C.

DIFFUSION EXPERIMENTS

The reducing substances were determined according to the method of Hagedorn and Jensen (1923); in the uncoagulated emulsion by analysis of 0.1 ml and in the overlay by analysis of from 0.2 ml to 1.0 ml depending on the expected amounts of reducing substances. In all cases the proteins were precipitated in the usual manner, and duplicate determinations were carried out. Obtaining data on the coagulated base material was not possible because of the difficulty of securing representative samples. The fats were measured by ether extraction; the uncoagulated emulsion, and the coagulated base were extracted by the method of Kumagava and Suto (1908). For analysis of the overlay a Friedrich's extraction apparatus was used and the entire overlay of a flask was extracted after slight acidification with sulfuric acid. The organic nitrogen was ascertained by means of Kjeldahl's method; in the uncoagulated emulsion by analysis of 2 ml and in the overlay, by analysis of 50 ml per flask. In all cases the overlay was filtered before analysis to remove particles of egg white.

The results of these tests are presented in Figs. 1 and 2, which show the concentrations in milligrams per cent reached by various substances in the overlay after specified periods of storage. Since the overlay contained 200 ml the concentration figures multiplied by 2 equal the amounts in milligrams. The reducing substances were calculated as glucose because Sørensen (1934) has shown that egg white contains 450 milligrams per cent of glucose. Our average figure was 597 milligrams per cent of reducing substances as calculated in the undiluted egg white. Whether the excess over Sørensen's figures for glucose is due to the occurrence of some other sugars in the uncombined state or to non-sugar types of reducing substances is not known. Egg white is known to contain glucose, mannose, galactose, and glucosamine. The total of all sugars according to Sørensen (1934) amounts to 700 milligrams per cent. However, mannose, galactose, and glucosamine appear to be bound to proteins and would consequently escape detection by the method of Hagedorn and Jensen. In any event it is apparent that the greater part of the material studied by us is glucose.

According to our data a base of 50 ml contained about 224 mg of reducing substances. In a theoretical diffusion equilibrium between base and overlay the concentration in the latter would be 90 milligrams per cent. Actually (Figs. 1 and 2), a steady state was observed in which the reducing substances reached a constant level of around 50 milligrams per cent. In other words, 95 to 105 mg, instead of a possible 180 mg, diffused out of the base. Two explanations of this discrepancy are as follows: The time during which the diffusion was followed may have been too

short to allow for complete equilibration. Some of the reducing substances of the uncoagulated base may have been indiffusible or have been destroyed during autoclaving of the base material. The first explanation appears untenable because the curves (Figs. 1 and 2) indicate that diffusion had come to a more or less complete standstill after 3 to 4 days storage at 37° C and 8 to 10 days at 10° C. The second explanation was tested in the following manner: Bases prepared and overlayed in the usual way were stored for one week at 37° C. The overlay was then drained off and analyzed for reducing substances. The bases were again overlayed with fresh

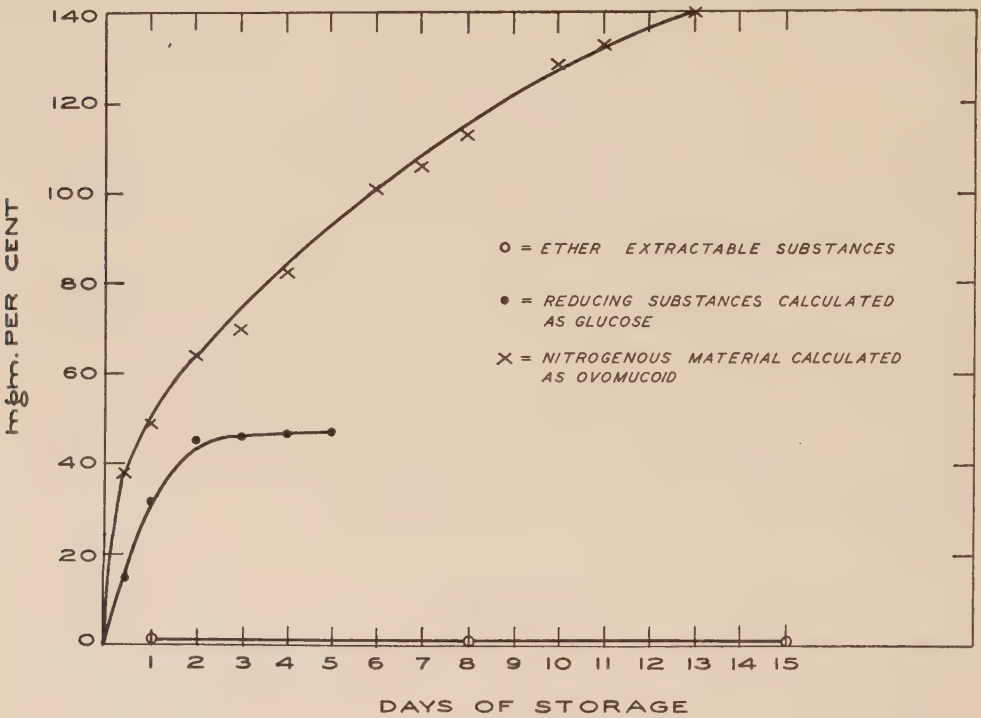

FIG. 1. Occurrence in milligram per cent of reducing substances (calculated as glucose), of nitrogenous material (calculated as ovomucoid) and of ether-extractable substances in the fluid phase of egg white medium during specified periods of storage at 37° C. Each point on the reducing substances curve is the mean value of 4 to 6 flasks, on the ovomucoid curve of 2 flasks and on the ether extractable substances curve of 4 flasks.

Locke's solution and again stored for one week and this procedure repeated over a period of 4 weeks. In a series of 8 flasks the total overlay per flask after one week contained 100 mg of reducing substances, after the second week, 28 mg after the third week, 12 mg, and finally after the fourth week, 10 mg. During four weeks therefore a total of 150 mg diffused out of the base. We may consider this the sum total of easily diffusible material per base. An equilibration between 50 ml of base containing 300 milligrams per cent of easily diffusible reducing substances and 200 ml of overlay would give in the latter a concentration of 60 milligrams per cent. This figure is in acceptable agreement with the figure actually observed. Indiffusibility or destruction of some reducing substances therefore accounts for the difference in amounts determined in the uncoagulated base on the one hand, and recovered from the overlay on the other.

The base was found to contain 120 to 140 milligrams per cent of ether extract (corresponding to 0.17 per cent of the undiluted egg white, or an average of 62 mg per base) regardless whether the material was analyzed before coagulation or after a week or two of storage. On the other hand, very little ether extractable material was found in the overlay. The absolute figure was about 2 mg per flask or one milligram per cent. Practically the same amount was found whether the analysis was made after one day or after 15 days of storage at 37° C (Fig. 1). Obviously therefore only small amounts of lipoids reached the overlay and the transfer mechanism was not diffusion. This result was anticipated since lipoids are insoluble in

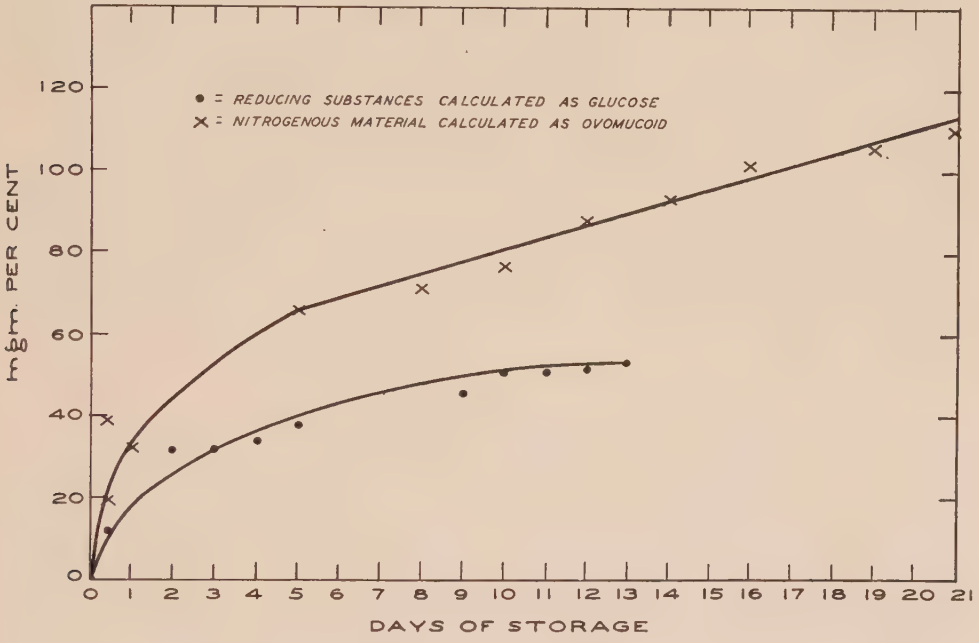

FIG. 2. Occurrence in milligram per cent of reducing substances (calculated as glucose), and of nitrogenous material (calculated as ovomucoid) in the fluid phase of egg-white medium during specified periods of storage at 10° C. Each point on the reducing-substances curve is the mean value of 5 flasks, on the ovomucoid curve of 2 flasks.

water unless present in the form of soaps and there is no indication that the latter occur in egg white. It appears therefore that the "sweating out" of lipoid droplets from the base during autoclaving may account for the amount appearing in the overlay. Owing to the small amount it was impossible to determine the chemical nature of the lipoids in the overlay.

Egg yolk contains much more lipoid than egg white. It appeared therefore of interest to study the ether extractable materials of whole egg for comparison with the studies on egg white. A whole egg base was found to contain from 8.51 to 9.09 per cent (average 8.89 per cent) and therefore 52 times as much lipoid material as egg white. The amount found in the overlay was low but nevertheless distinctly greater than in the egg white medium. In the whole-egg overlay after one day of storage at 37° C there was 8 mg per flask, after one week, 7 mg and after two weeks, 8 mg or an average of around 4 milligrams per cent. And thus, although the amount

in the overlay was four times that occurring in egg white overlay, the figures suggest very strongly that no diffusion was involved.

Egg white contains 5 known protein fractions; ovalbumin, ovoglobulin, ovomucin, conalbumin, and ovomucoid. The first four are heat coagulable, the last non-heat coagulable, and therefore, diffusible from an inspissated base. The nitrogen found in the overlay was calculated as ovomucoid nitrogen. According to Sørensen (1934) ovomucoid contains 12.63 per cent nitrogen; the conversion factor used in our calculations was 7.9.

As indicated in Figs. 1 and 2 diffusion of ovomucoid takes a longer time to reach a standstill than was the case with reducing substances. As a matter of fact it had not ceased at the termination of the experiments. How far it had progressed can be shown by a simple calculation. The ovomucoid nitrogen amounts, as shown by Sørensen (1934), to 10.63 per cent of the total nitrogen of all egg-white proteins. One egg-white base contains, according to our determinations, an average of 622 mg nitrogen. Its ovomucoid fraction corresponds to 66 mg nitrogen or 523 mg ovomucoid. Complete equilibration between base and overlay would lead in the latter to a nitrogen concentration of 26 milligrams per cent, which equals 210 milligrams per cent ovomucoid. The observed figure after storage for two weeks at 37° C was 17.8 milligrams per cent which equals 140 milligrams per cent of ovomucoid. The trend of the curves (Figs. 1 and 2) indicates that complete equilibration would require a long time.

SPONTANEOUS OXIDATIONS

Studies on growth promoting properties of egg-white medium for *E. histolytica*-organism *t* when freshly prepared and after storage for two weeks or longer at 37° C and 10° C have shown that stored cotton stoppered medium lost amoeba-producing properties and that rubber stoppered medium retained these properties especially when the residual oxygen was absorbed by alkalinized pyrogallol (Rees and Reardon, 1945). It seemed likely that in the cotton-stoppered flasks some growth-promoting factor was rendered inactive through oxidation. Since, however, the exact nature of growth-promoting factors of egg white is unknown the problem is not amenable to direct approach. It was therefore studied in an indirect manner by ascertaining whether large-scale oxidations occur. If this were the case the oxidations could obviously be connected with any of the main constituents of egg white as well as with those present in only small amounts; and even the occurrence of only small-scale oxidations would be in agreement with the above-mentioned theory that oxidation affects some growth-promoting substances.

The experiments are summarized in Table 1 in which the air content of flasks under indicated conditions was analyzed for oxygen and carbon dioxide. It will be noted that analysis was made over diphasic medium and over dry egg-white base prepared with and without egg shell. The half portions of base and overlay of the diphasic medium were necessary to permit sufficient air for analysis. Before overlaying the medium Locke's solution was saturated with air by shaking for 3 hours at 10° C. The analyses were conducted in a van Slyke manometric apparatus. One control series was run to determine the amounts of the respective gases absorbed in and given out from Locke's solution, and the other control series with air-filled flasks to ascertain whether progressive absorption of gases occurred in the rubber stoppers.

The figures for carbon dioxide show a higher concentration of this gas in series 1 in air above the diphasic medium than in the other series of flasks but in other

respects the comparative figures for carbon dioxide in the different series and for different storage periods fail to show consistent trends.

On the other hand the figures for oxygen content show a slight decrease after 8 days storage for all series containing egg white, the entire observable changes occurring during the first week of storage. For example, omitting the figures for one-day storage, the mean for series 1 is 19.86 per cent and for series 4 containing only Locke's solution the mean is 20.64 per cent. Since the gas space amounted in both cases to 125 ml about 1 ml of oxygen was used up in the oxidations that were directly connected with the medium. In these calculations no account was taken of the gases dissolved in the fluid or in the base. When computed on the same basis as above the mean values of series 2, 3, and 5 are 19.85, 20.11, and 20.70 per cent, respectively, showing oxygen losses of 1.7 ml and 1.2 ml per flask, respectively, of series 2 and 3. The order of magnitude is therefore the same as in series 1 and as

TABLE 1.—*Oxygen and carbon dioxide content of air above egg-white medium and Locke's solution in Florence flasks; and of air-filled flasks after specified periods of storage at 10° C*

Series number	Kind of material in flasks	Storage period days	Oxygen per cent	Carbon dioxide per cent
1	25 ml egg-white base, 100 ml overlay	1	20.35	0.50
		8	19.85	0.72
		15	19.85	0.75
		22	19.87	0.69
2	50 ml egg-white base, 3 gm egg shell	1	20.35	0.27
		8	19.75	0.27
		15	19.76	0.28
		22	19.83	0.23
3	50 ml egg-white base, without egg shell	1	20.36	0.33
		8	20.11	0.16
		15	20.02	0.23
		22	20.21	0.17
4	125 ml Locke's solution	1	20.59	0.13
		8	20.53	0.14
		15	20.76	0.21
		22	20.63	0.24
5	Air-filled flask	1	20.66	0.23
		8	20.75	0.14
		15	20.62	0.11
		22	20.73	0.20

previously indicated the figures are in agreement with the theory that oxidation of some substance affecting the growth of *E. histolytica* may have occurred.

DISCUSSION

The experiments reported upon in the preceding sections show that relatively large amounts of reducing substances, essentially glucose, and of nitrogenous material, essentially ovomucoid, diffuse from an egg-white base into the overlay, but that very little ether-soluble material occurs in the latter. The diffusion of the reducing substances is rapid, that of the nitrogenous material slower.

Since *E. histolytica* and its associated bacteria obviously derive food from the substances present in the overlay this diffusion has to be taken into account if uniform external conditions are to be established. For example, the overlay in a flask, stored for 24 hours at 37° C contains only one-third of the nitrogenous material of that of one stored at that temperature for 2 weeks. Amoebae inoculated into both flasks will grow under quite different external conditions. Similarly, a flask stored for one week at 37° C will be a quite different medium from one stored for an identical period at 10° C. In comparative studies it seems advisable to use culture flasks of identical age that have been stored at the same temperature. If this is done the

external conditions are very uniform. The variations between flasks analyzed at one time were small and this applied to all substances studied. The curves presented in Figs. 1 and 2 can be used to make definite statements about the concentration of organic material available to organisms at any time covered by them, provided that the ratio between base and overlay is the same as in our experiments and that the temperature of storage was either 37° C or 10° C. Any quantitative change in the materials used, any change in temperature, or the use of different flasks (which would change the surface area of the egg-white base) will change the rate and amount of diffusion.

In all experiments in which older flasks are to be used, the media should be protected from contact with air during the time of storage, but clear proof that no large-scale oxidations occur when egg-white media are stored at 10° C has been presented.

SUMMARY

Egg-white medium dispensed in Florence flasks was stored at 37° C and 10° C for periods up to 20 days and analyzed for diffusion of reducing substances, fats, and nitrogenous compounds. The reducing substances were calculated as glucose and the nitrogenous compounds as ovomucoid. Diffusion of reducing substances was rapid, coming almost to a standstill after 2 and 8 days at the respective temperatures. Diffusion of ovomucoid was considerably slower, and although most rapid during the first 8 days, it was still in progress after 20 days. Only a trace of lipoid was demonstrable in the overlay and there was no evidence indicating diffusion.

There was evidence of small-scale spontaneous oxidations in stored medium. A decrease of oxygen content amounting to 1 ml per 125 ml of air confined over diphasic medium and non-overlayed base was demonstrated. These data are in agreement with a theory that some component of egg white contributing to the growth of *E. histolytica* may be rendered inactive by oxidation. Some increase in carbon dioxide content of air over stored medium occurred but comparative figures show no consistent trends.

These data may be utilized in standardizing medium for the cultivation of *E. histolytica.*

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OBSERVATIONS ON *DOGIELELLA RENALIS,* N. SP. (ASTOMATA, CILIOPHORA) FROM THE RENAL ORGAN OF *PHYSELLA* SP.

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Examination of fresh-water gastropods from some of the marginal swamps and tributaries of Lake Washington, adjacent to the University of Washington campus, has disclosed not only the intense larval trematode infestation which might be expected, but also the annelid, *Chaetogaster,* and a considerable protozoan fauna, chiefly of flagellates and ciliates. Among the last, one, a species of ASTOMATA infecting *Physella* sp., has proved of particular interest.

On several occasions all adult snails collected from one pool in the University of Washington Arboretum have been found infected; the ciliate being present in great numbers in the renal organ, with a few specimens occasionally found in the mantle cavity. In one other area, a part of the littoral swamp about one-half mile from the first station, a moderate number of infections have been met with. Hosts from other parts of the same drainage system from which collections were made, although frequently encountered, consistently lacked the infection.

Detailed study of a large number of fixed and living ciliates indicated that this astome is certainly a member of the family ANOPLOPHRYIDAE as amended by Cheissin (1930). The subfamilial relationship is less evident as the present species has characters in common with the PEREZELLINAE and with the ORCHITOPHRYINAE. Among the subfamilial, differential characters mentioned by Cheissin only one, the presence or absence of a contractile vacuole, can be considered as distinguishing the PEREZELLINAE from the ORCHITOPHRYINAE. Since the species from *Physella* does possess a contractile vacuole it seems desirable to consider it a member of the former subfamily. Among the genera of this subfamily the species most closely resembles *Dogielella* Poljansky, 1925, with which it shares the elongated, pyriform shape; uniform, longitudinally arranged cilia; rounded, centrally located macronucleus; and granular endoplasm. It is set off from *Perezella* Cépède, 1910, by the absence of a ventral attachment surface; and from *Perseia* Rossolimo, 1926, by the possession of a rounded rather than a greatly elongated macronucleus. Since certain characters to be described distinguish the *Physella* ciliate from reported species of *Dogielella* it is regarded, here, as new to science and designated *Dogielella renalis,* n. sp.

Specific diagnosis of Dogielella renalis: This is an elongated, astomatous ciliate found in great numbers in the cavity of the renal gland of *Physella* sp. The anterior end is more or less pointed, the posterior bluntly rounded, the body extremely plastic and variable in shape (Fig. 9). The mean measurements of fifty specimens were: length, 110.8 μ (61.2–183.6); breadth, 41.5 μ (27.2–81.6); macronuclear diameter, 18.7 μ; micronuclear diameter, 4.6 μ; expanded contractile vacuole, 15.1 μ; cilia, 7.3 μ. The macronucleus is approximately spherical, contains finely granulated chromatin, and lies in the middle of the body; the micronucleus is spherical, it may be homogeneous or granular, and its position is variable. The cilia are uniform in length and are arranged in about forty longitudinal rows. These rows contain fewer cilia over the elongated, anterior part of the body, and terminate posteriorly at the margin of a small, triangular, non-ciliated area (Fig. 3). The cytoplasm is differentiated into ectoplasm and endoplasm; the former containing ciliary basal granules, fibrils, and Golgi granules; the endoplasm is packed

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with a variety of granules, and, anterior to the macronucleus, contains the contractile vacuole and its accessory vacuoles.

Source: Littoral drainages of Lake Washington.

Host: Physella sp., renal organ.

The size, alone, would indicate that this ciliate differs from *Dogielella minuta, D. virginia,* and *D. globulifera.* This character is not adequate to distinguish it from *D. sphaerii* since, although the typical specimen of *D. renalis* is considerably larger, there is overlapping. The pointed anterior and rounded posterior ends of *D. renalis* are almost a reversal of the normal proportions of *D. sphaerii.* Perhaps the most adequate differential characters are the anterior position of the contractile vacuole and the habitat. In all other species the vacuole assumes a posterior and terminal position, and all other species are reported as parasites of parenchyma while *D. renalis* parasitizes the cavity of a viscus.

The fact that neither *Dogielella renalis* nor *Perseia dogieli* possess a terminal contractile vacuole renders inaccurate the sub-familial diagnosis of Cheissin:

Unterfam. Perezellinae (Cépède) Cheissin. Durch terminal gelagerte kontraktile Vakuole und durch runden oder ovalen Kern charakteriziert.

In order to include these species the following modification is suggested:

Subfamily Perezellinae (Cépède). Characterized by the possession of one or more contractile vacuoles, and by round to elongate macronuclei.

The reported species within the subfamily may be differentiated:

I. Macronucleus round or ovoid (II)
  A. Cysts unknown (B)
    1. Contractile vacuole terminal (2)
      a. Anterior end broader than posterior (b)
        AA. Parasite of lamellibranch ..... *Dogielella sphaerii*
        BB. Parasite of turbellarian ..... *D. globulifera*
      b. Anterior end narrower than posterior
        AA. Length less than 30 μ ..... *D. minuta*
        BB. Length greater than 40 μ ..... *D. virginia*
    2. Contractile vacuole anterior ..... *D. renalis*
  B. Cysts formed ..... *Perezella pelagica*
II. Macronucleus elongated ..... *Perseia dogieli*

OBSERVATIONS ON STRUCTURAL DIFFERENTIATIONS

The locomotor system.—In attempting to work out the details of the locomotor system, observation of living ciliates has been supplemented by study of fixed preparations. Several silver methods have been tried, of which one, involving preliminary brief fixation by osmium tetroxide vapor followed by exposure to 2% silver nitrate on the slide, has been by far the most satisfactory for the demonstration of pellicular structures. In addition, a number of renal glands have been dissected out and fixed, whole, in Zenker's solution or in Schaudinn's solution. After sectioning, these were stained with Mallory's triple stain, with Heidenhain's haematoxylin, or with Harris' haematoxylin. Details of the inner fibrillar complex were revealed by the haematoxylins, but the triple stain proved of little value.

The body is clothed by approximately forty rows of paired cilia; each row occupying a longitudinal furrow of the pellicle. At two points the rows are interrupted: in the anterior half of the body two or three rows are discontinuous over the permanent point of discharge of the excretory vesicle; at the posterior end of the body all rows terminate at the margin of a small, triangular, non-ciliated area (Fig. 2, 3).

Over most of the body the ciliary basal granules appear, in silver preparations, to be nearly touching one another. This is probably due to thickening of the granules by silver deposit as they appear much larger by this than by other methods. At the anterior end of extended specimens the granules may be several times as far apart as in contracted ciliates, or as in the mid-portion of the body of the same animal. On first inspection, the silver preparation produces a strong impression of a series of basal granules lying in a sub-pellicular strand. This is not confirmed by further study and comparison with sectioned material. The longitudinal strand is undoubtedly a depressed thickening of the pellicle through which the cilia emerge from a series of basal granules internal to the pellicle. By reason of their situation the longitudinal, rib-like thickenings must reinforce and support the body. That they are somewhat elastic is shown by their elongation and variation in thickness when the anterior end of the body is extended. Whether they play any active part in changing the body form has not been determined.

Sections through the body wall, when stained with haematoxylin, reveal additional structural detail (Fig. 4). The cilia arise in pairs from basal granules lying wholly internal to the pellicle but close to the under-surface of the longitudinal ribs. These granules are connected by delicate fibrils which course through the ectoplasm, and appear to have a distribution identical to that of the pellicular ribs. Whether the fibrils are interrupted over the excretory pore has not been observed. At irregular intervals transverse fibrils link basal granules in adjacent rows. Finally, a loose mesh of cytoplasm ramifies between the pellicle, the granules, and the fibrils, uniting them with the internal body structure.

In view of the widely differing interpretations which have been placed upon various ciliate fibrillar systems and structures, there can be little value in attempting a rigid, functional analysis of the structures observed in *Dogielella renalis*. Suffice to say that the pellicular ribs are admirably suited to serve as an elastic support to the body, while the inner, fibrillar complex has an arrangement which would be well adapted to a conductive function.

Cytoplasmic inclusions.—Although no extensive study of the cytoplasmic makeup has been undertaken, it is evident that several distinct types of granules are present. After silver impregnation, blackened granules, approximating 0.25 μ in diameter (Fig. 5), are found in great numbers, chiefly in the ectoplasmic layer. Their distribution and staining behavior suggest that they are comparable to the ectoplasmic Golgi granules of MacLennan (1936). In addition to these, silver impregnation reveals numerous granular complexes in the endoplasm. The latter consist of one or more argentophile granules associated with a larger mass which is weakly argentophile in the cortex only (Fig. 6). Such bodies average 2 μ in diameter. After non-acid Zenker fixation they stain uniformly mauve with haematoxylin, and they are well preserved in preparations fixed with Altmann's fluid. It seems probable that the granular, argentophile portion represents mitochondria while the non-staining mass is the specific substrate upon which they are working.

Reserve material, in this species, is found chiefly in the form of lipoid globules. Sections of ciliates, fixed *in situ* with potassium bichromate-osmium tetroxide mixtures, are frequently filled with droplets of blackened material. Accepting the contention of Gatenby that material which blackens freely with osmium tetroxide in the presence of potassium bichromate belongs to the true fats, it is evident that the lipoid

of *D. renalis* is of this character. Although the lipoid may be found throughout the body it is usually present in greatest quantity posterior to the macronucleus (Fig. 7, 8). In this area the largest globules are concentrated, often reaching a diameter of 6 μ. A few attempts to demonstrate paraglycogen, by the use of iodine–sulphuric acid on the living ciliate, have failed. No specific granules were stained, although a diffuse, brownish color was imparted to the cytoplasm.

PHYSIOLOGICAL OBSERVATIONS

Host-parasite relations.—Due to concurrent infection with larval trematodes it has been impossible to establish the pathological significance of *Dogielella renalis* for its host. Abnormalities in snails with the mixed infection were not demonstrably greater than those found when only larval trematodes were present. Since the observed abnormalities in both cases were wholly local, and consisted of tissue damage of a character usually considered due to pressure, there are not adequate grounds for presuming the ciliate to be either innocuous or contributory to the injury.

From the point of view of the ciliate the parasitic relationship is fixed and obligatory. Not only is the organism structurally limited to osmotic feeding, but the forms ordinarily encountered survive for very short periods outside the host. Whether released into culture water, various dilutions of saline, or tissue fluids and secretions of the host, they soon degenerate; commonly showing marked changes in the macronucleus, and cytoplasmic vacuolization, within as little as ten or fifteen minutes.

The transmission of infection.—The degree to which the parasite is dependent upon its host suggested that the mode of transfer of the infection might be of interest. As casual observation offered no information on this point, a few experiments were undertaken to attempt to determine how this very delicate ciliate passed from host to host.

Eggs, obtained from snails from infected groups kept in the laboratory, were inspected with a dissecting binocular to eliminate the possibility that the ciliate was transmitted in the egg. No ciliates were ever observed in this situation. The egg-masses were then washed and allowed to hatch in separate vessels. After hatching, young snails were examined at intervals to preclude possible infection developing from obscure and unrecognized ciliates in the egg. No infection was ever detected. It was concluded, when the residual young snails reached four weeks of age, that snails so reared were free from infection. Accordingly, these snails, or others similarly reared, were used in the following tests.

Experiment 1.—Several heavily infected kidneys were dissected out and broken up in a Syracuse watch dish in the fluid and mucous from the snails from which they had been taken. Twelve young snails were added to the suspension and allowed to remain until no living ciliates could be detected. They were then removed, washed free of gross debris, and established in clean culture vessels. Examination of one-fourth of the young snails during each of the succeeding four weeks failed to reveal any infection.

Experiment 2.—Several infected kndneys were macerated and added to a culture of young snails. No living astomes were observed after one hour. Examinations of samples at weekly intervals for four weeks disclosed no infections.

Experiment 3.—A culture of young snails was added to a culture from an in-

fected source. After one week the adults were removed and examined: of twelve snails, eleven were infected, eight heavily. Examination of young snails at the same time revealed no infections nor were any found in samples taken during the ensuing three weeks.

Experiment 4.—The failure to obtain infection when a watery medium intervened between parasite and potential host suggested that corporal contact between actual and potential host might be essential for transference of the infection. Accordingly, adult snails were obtained from a stream in which infection had not been previously encountered. A number of these were examined, and no infections were found. The remainder were marked with indelible ink and placed in a crowded culture with adult snails from an area of heavy infection. Copulation and other close contacts were frequent. At the end of ten days four marked and four unmarked snails were examined: no infection was found in the former; three of the latter contained large numbers of *Dogielella*. Additional samples, taken at weekly intervals for the following four weeks, revealed no infection in the marked snails, but a constant, high level of infection in their associates.

The absence of detectable ciliates from egg-masses produced by snails infected with *Dogielella renalis,* and the consistent absence of ciliates from young snails reared from these egg-masses, indicates that infection through the egg is not usual. Failure to find the ciliate in any other part of the body than the renal organ and the mantle cavity is further indication that the egg can scarcely be the customary agent of transfer.

Failure of the typical ciliate to survive in a watery medium beyond the briefest periods renders indirect transfer from snail to snail improbable, unless the existence of a resistent stage be postulated. Experiments 1, 2, and 3, in which presumably susceptible offspring of an infected population were exposed to living ciliates without becoming infected, are in agreement with this presumption.

Due to the fact that there was no check on the susceptibility of the uninfected snails used in Experiment 4 this test cannot be considered as significant as the others. However, since both tests 3 and 4 were essentially similar in providing opportunity for close contact between infected and uninfected animals over a period of time, they may be considered, together, to suggest that proximity of the actual and the potential host does not enhance the chance of passage of the vegetative ciliate from one animal to another.

Thus, observation and trials cast considerable doubt on the transmission of infection either through the egg or through more or less indirect passage of the typical, vegetative ciliate. Since adequate demonstration of the transmission of parasitic protozoa through the host's egg is rare, and largely limited to the Sporozoa, this finding is scarcely surprising. It is of interest to note, in connection with failure to transmit the vegetative ciliate, that somewhat similar behavior has been noted in related species. Although *Orchitophrya stellarum* survives for long periods in sea water, Cépède (1910) failed entirely to obtain transfer of the infection. Likewise, although the infections observed were massive, he encountered only three infected hosts among some six thousand examined. In view of the common crowding together of starfish, it is improbable that this low incidence could have occurred were infection established by contamination. Poljansky (1925) noted the extreme rarity of infection of immature *Sphaerium,* which had not left the brood pouches of the

mother, with *Dogielella sphaerii,* and considered it evident that this ciliate did not pass normally from mother to offspring either through the egg or during development. Since *D. sphaerii* quickly degenerated in the environmental water he postulated the existence of a more resistant stage to account for the seasonal rise in infection which he observed. That this resistant form is a cyst is rendered doubtful by the fact that at no time did he observe cysts or their production.

Although circumstances rendered it impossible to determine the question experimentally, certain field observations suggest that the infective form may be a product of conjugation. Initial collections from the Arboretum station in the fall and early winter of 1941–1942 showed an infection level in adult hosts approximating 90%. Very young snails were not examined at this time. No further collections were made until the spring of 1943 when the snail population was found to be greatly reduced. The following collections and observations were made at that time:

Date	*Adults*	*Young*	*Infections*	*Remarks*
3/17/43	13	2	10 adult	
4/19/43	14	Many	9 adult	No infection in fifty young snails examined.
5/7/43	6	Many	5 adult 1 young	All adult infections showed conjugation.
5/13/43	2	Many	1 adult 5 young	Conjugation in adult. Fifty young snails examined.
5/19/43	3	Many	2 adult 24 young	No conjugation observed. Fifty young snails examined.

No young hosts were found to harbor infection until conjugation appeared in adult hosts. From this point, the infection level in young hosts steadily climbed. In this connection it is pertinent to mention the observations of Poljansky on *D. sphaerii.* He found the following infection levels: May, 15%; June, 66%; July, 88%; November, 25%. Conjugation was met with from June until the middle of August. Thus, the period of great increase in infection level coincides, in *D. sphaerii* as in *D. renalis,* with the period of conjugation. The later decline of infection level in *Sphaerium* may well be correlated with the reported sudden destruction of a large proportion of the infected individuals towards the end of July when the infections were noted to be extremely heavy.

One might postulate that in conjugation particularly resistant forms are produced, or that a state of unusual physiological vigor permits transfer of infection. While the mechanism involved is completely obscure, the rapid, seasonal rise in infection level on the part of an organism which is ordinarily highly intolerant of its extra-hostal environment can scarcely be disregarded. That this transfer of infection coincides with another physiologically important reaction strongly suggests a relationship to exist between the two. Investigation of the resistant powers of the peculiar, large, bimacronucleate exconjugants of *D. sphaerii* (Poljansky, 1926) might be of interest in this connection.

SUMMARY

Dogielella renalis, n. sp., is described from the renal organ of *Physella* sp. It differs from previously reported species in size, in contractile vacuole position, and in habitat.

A few attempts to establish experimental infection suggest that normal transfer is neither through the host's egg nor by means of the vegetative ciliate. Field ob-

servations make it seem probable that conjugation is a necessary prelude to the attainment of the infective state.

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Explanation of Plate

Unless otherwise stated all figures have been drawn with the aid of a camera lucida. Stated magnifications are those at which the figures were drawn; reduction in reproduction is indicated by a five centimeter line accompanying the plate.

Fig. 1. *Dogielella renalis,* entire animal, showing nuclei and contractile vacuole with accessory vacuoles. Flemming fixation, Heidenhain's haematoxylin. ×1000.

Fig. 2. Entire animal, showing pellicular structure. Osmius tetroxide vapor and silver nitrate. ×1000.

Fig. 3. Posterior end. Technique as in 2. ×2500.

Fig. 4. Semi-diagrammatic reconstruction of a portion of the locomotor organelles. ×8000.

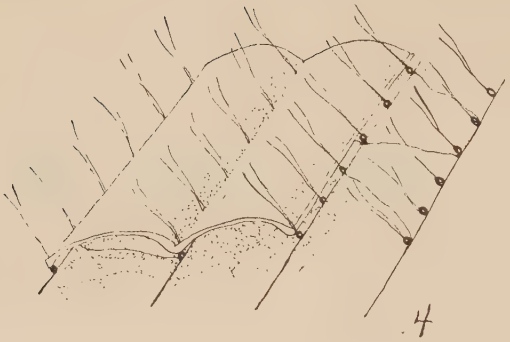
Fig. 5. Ectoplasmic Golgi granules. Osmium tetroxide impregnation. ×2500.

Fig. 6. Endoplasmic argentophile bodies. Osmium tetroxide vapor and silver nitrate. ×2500.

Fig. 7. Section through anterior end of ciliate, showing scanty, lipoidal inclusions. Potassium bichromate–osmium tetroxide fixation. ×1000.

Fig. 8. Section through posterior end of same ciliate, showing massive lipoid reserve. ×1000.

Fig. 9, (a–h). Ciliates fixed with osmium tetroxide vapor, showing plasticity and variability of form. ×250.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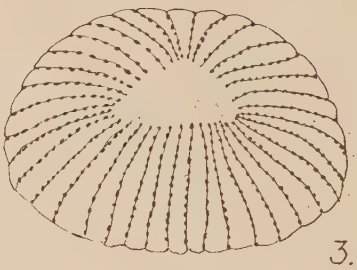
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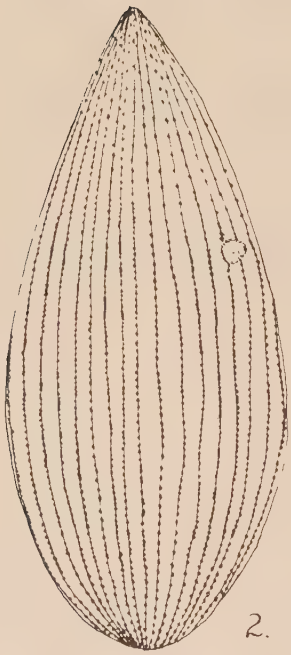
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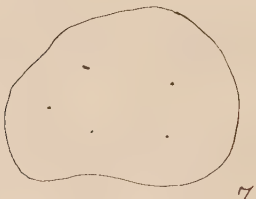
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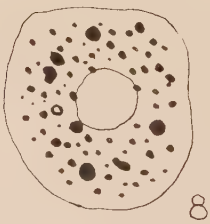
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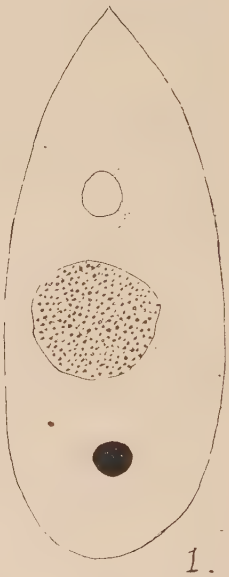
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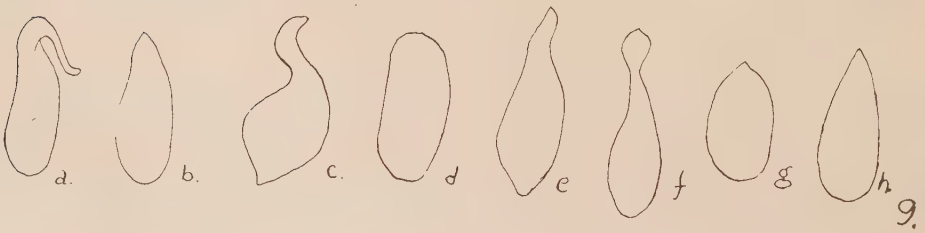

9.

OTTO FUHRMANN

Jean G. Baer
Université de Neuchâtel

By the death of Otto Fuhrmann, which occurred on January 27th 1945, after a short illness, the American Society of Parasitologists has lost one of its most prominent foreign honorary members.

Born on the 1st of April 1871 in Basel where his father occupied the position of procurist to the well-known firm of Geigy, Fuhrmann entered the university of his native town at the age of eighteen and became the pupil of such famous men as Rütimeyer, the comparative anatomist, and Zschokke, who was himself a former pupil of Leuckart. While preparing his thesis, Fuhrmann spent a semester in Geneva where he came under the influence of Cart Vogt which was to be decisive in diverting the young student's attention to the parasitic flat-worms. After having obtained his Ph.D. in Basel, Fuhrmann returned to Geneva as assistant to professor E. Yung, Carl Vogt's successor in the chair of Zoology and one year later, in 1895, he accepted a call from the University of Neuchâtel where he remained until his retirement in 1941, as Professor of Zoology and Comparative Anatomy.

From the very beginning, Fuhrmann's work was of an outstanding quality and his first paper on Cestoda, published in 1895, is still in advance of many of the more modern descriptions. Specializing principally in avian tapeworms, he reexamined and redescribed almost all of the collections made by Rudolphi and Diesing which had been deposited in the museums of Berlin and Vienna. Eight years of patient research furnished Fuhrmann with the basis for establishing his classification of the Cyclophyllidea which has stood the test up to the present day. Prior to his preliminary note published in 1907, the Cyclophyllidea were considered by Max Braun as forming a single family divided into nine subfamilies whereas Fuhrmann split them up into ten distinct families and nine subfamilies. Of the sixty-six genera, twenty were new to science and thirty-one were redescribed from the original specimens. One of the merits of this new classification was to bring out the fact that three of the families, *viz.*, Anoplocephalidae, Davaineidae and Dilepididae appear to have evolved on parallel lines as shown by the structure of the uterus. The latter is persistant in the Anoplocephalinae, Ophryocotylinae and Dilepidinae; it breaks down into egg capsules in the Linstowinae, Davaineinae and Dipylidinae and a paruterine organ appears in the Thysanosominae, Idiogeninae and Paruterininae. Whereas the older helminthologists based their systematic divisions chiefly on scolex structure, Fuhrmann stressed the importance of the arrangement of the reproductive organs. Developing Krabbe's theory of parallel evolution between the bird hosts and their tapeworms, Fuhrmann showed that host-specificity in this group of helminths attains a very high degree. This theory is the guiding thread throughout his entire work and is to be found in its most perfect form in his "Cestoden der Vögel" published in 1908 and of which a new and revised edition appeared in 1932 under the title "Les Ténias des Oiseaux." A complete list of Fuhrmann's papers has been published elsewhere (1945, Bull. Soc. neuch. Sc. Nat. 69: 159–165), but amongst these a special mention must be made of his con-

OTTO FUHRMANN

tributions to Kükenthal's *Handbuch der Zoologie,* of which he wrote the chapters on the TREMATODA and the CESTODA. Although written in German, these parts are remarkably clear and concise and no student of helminthology should embark on research work without having first read through these chapters.

Although reputed abroad as an helminthologist, Fuhrmann was better known in Switzerland as an ichthyologist as well as an authority on fish-breeding. Several improvements in the breeding of Salmonides were introduced by Fuhrmann after his visit to the States in 1907 and have been adopted by most of the Swiss establishments.

The writer had the privilege of becoming Fuhrmann's pupil nearly twenty-five years ago and has ever since been constantly associated with him. He the more so feels how great a loss he has incurred by the death of such an inspiring teacher to whom he owes the foundations of his scientific career. Socially, Fuhrmann was of a retiring nature but became transformed as soon as he entered his laboratory. To his younger students, his knowledge of zoology and comparative anatomy appeared almost uncanny, it was, however, the outcome of a lifetime of hard work and extensive reading. Distrustful of general theories, he always brought his students back to the basic facts, thus leaving them to choose the theories to adopt. His enthusiasm when one of his pupils found some new combination of tapeworm characters or a new form of fresh-water invertebrate, was almost boyish and in consequence the atmosphere of his institute was anything but formal. Our admiration for Fuhrmann, and his fatherly way of helping us over a particular difficulty, created a family atmosphere which was most congenial to research work. Closing hours were unknown, and there were always seven days to the week for research! With Fuhrmann disappears one of the last great helminthologists of the pioneer generation to which we owe so much of our present knowledge. His life-work remains the greatest tribute that could be paid to his memory.

TRICHURIS PEROMYSCI N. SP. FROM *PEROMYSCUS CALIFORNICUS,* AND FURTHER NOTES ON *T. PEROGNATHI* CHANDLER, 1945

In a recent paper (1945, J. Parasitol. **31**: 284) the writer reported on four species of *Trichuris,* three of them new, from rodents collected on the Hastings Natural History Reservation in California. Each rodent represented harbored a different species, and the marked host specificity for members of this genus was pointed out. Subsequently a series of specimens from another rodent, *Peromyscus californicus,* were made available from the same locality through the kindness of Mr. J. M. Linsdale. These represent still another new species, for which the name *Trichuris peromysci* is proposed, and the following description given:

Trichuris peromysci n. sp.

Male 14.7 to 31.8 mm long; posterior part of body 5.5 to 11.5 mm long, anterior part 9.2 to 20.8 mm long, the ratio being from about 3 : 5 to 1 : 2. Maximum diameter of posterior part 400 to 480 microns, of middle of anterior part about 100 to 110 microns. Posterior end of body rounded. Spicule 0.86 to 1.4 mm long, 28 to 32 microns broad at tip, broadening to about 50 to 55 microns about 250 microns from tip, then narrowing to about 35 to 38 microns, except at proximal end, where it flares to about 60 microns. Spicular sheath, when fully extruded, bell-shaped, projecting from body 260 to 360 microns, the exserted part covered with blunt spines distally, short sharp ones proximally. Spicular tube joins cloacal tube 375 to 525 microns from posterior end. Entire cloacal tube 1.2 to 2 mm long, the portion proximal to junction of spicular tube being, therefore, two to three times length of portion distal to this junction. Vas deferens over twice as long as ejaculatory duct. Testis irregularly lobulated, ending at proximal end of cloacal tube.

Female 23 to 41 mm long; posterior part of body 9 to 17 mm long, anterior part 14.3 to 24.4 mm long, the ratio being about 2 : 3. Maximum diameter of posterior part 550 to 625 microns, diameter of middle of anterior part 100 to 120 microns. Vulva just behind junction of esophagus and intestine, without marked prominence. Ovejector narrow, thick-walled, 0.7 to 1.4 mm long, widening abruptly into uterus. Anus almost terminal. Eggs 87 to 92 microns by 40 microns.

Location: Caecum.
Host: Peromyscus californicus.
Locality: Hastings Natural History Reservation, California.
Type and allotype: U. S. National Museum, Helm. Coll. No. 45884.

This species differs from species previously described from North American rodents by the following combination of characters: anterior portion of body much longer than posterior portion; tail of male not bilobed; anus of female almost terminal; and very large size of eggs, equalled only in *T. neotomae.*

Specimens of a *Trichuris* from *Perognathus penicillatus* from near Tucson, Arizona, were kindly supplied by Dr. Wanda S. Hunter. These conform with *T. perognathi* Chandler, 1945 from *P. californicus,* except that in the Arizona specimens the vulva is not situated on a cuticular evagination. Apparently this evagination of the ovejector is not a constant character, but is due to the manner in which the specimens were killed. The junction of the ejaculatory duct and vas deferens, not seen in the California specimens, is seen in the Arizona specimens to be well behind the middle of the posterior portion of the body, making the vas deferens about two and one-half times as long as the ejaculatory duct.—Asa C. Chandler, *Rice Institute, Houston, Texas.*

THE RACCOON, A NEW HOST FOR *MICROPHALLUS* SP., WITH ADDITIONAL NOTES ON *M. OVATUS* FROM TURTLES

On September 24, 1943, a raccoon (*Procyon lotor*) was brought me for parasitological examination by Dr. Lawrence E. Hicks. It had been killed on the road the previous evening in Pickaway County, Ohio. In addition to specimens of *Ascaris columnaris* and *Physaloptera rara,* hundreds of very small trematodes were found, distributed throughout the small intestine. The trematodes were very difficult to locate and separate from the mucus and debris in the intestine, so no effort was made to count them.

The specimens were killed and fixed by immersion in hot 10% formalin and permanent slides were made of stained specimens. Several specimens were also sectioned, and a total of more than 250 specimens was examined.

These trematodes belonged to the genus *Microphallus,* but differed from *M. opacus* (Ward,

1893) Ward, 1901, and *M. ovatus* Osborn, 1919, previously described from North America, in the following ways: Body 0.726 ± 0.145 mm long, and 0.439 ± 0.097 mm wide (100 specimens measured). Integument of anterior half of body spinose; spines recurved, about 0.0014 mm long, decreasing in size posteriorly and disappearing near middle of body. Vitellaria lobate, with 3 to 7 distinct lobes; lobes more compact than those of the other two species. Uterus very voluminous, filling body posterior to the acetabulum. Egg averages 0.025×0.012 mm. Besides marked differences in measurements, the specimens differed morphologically from the previously described species, although the general appearance is the same. The seminal vesicle is relatively larger and more transverse, and the mass of the prostate is relatively smaller. The vitellaria are more anterior, and the vitelline reservoir is larger. The intestinal crura, while variable, are relatively longer and more definitely formed. The anterior half is spinose, while *M. opacus* is described as spineless, and *M. ovatus* is described as spinose throughout. No variation was noted in the arrangement of the spines in these specimens.

The status of the species of this genus in North America is indefinite. It is of interest to note, that while fish have been considered the natural host of *M. ovatus* [considered identical with *M. opacus* by Strandine (Trans. Amer. Mic. Soc. **62**: 293–300) and others], this fluke appears to reach its maximum development in a turtle, *Graptemys geographica*. In 355 specimens of *M. ovatus* from 4 *Graptemys*, collected in central Ohio, the gross appearance of the worm was so different as to give the impression of a different species. The uterus was very voluminous and occluded from view all the other reproductive organs, as is the case with the specimens from the raccoon. The uterus stood out in the living specimen; was very dark in color, and extended anteriorly to the acetabulum. In these specimens the anterior end was usually much more attenuated, and the shape was, therefore, not oval, as has previously been considered typical. The eggs of these specimens measured 0.029×0.016 mm, which is intermediate between the measurements given for *M. ovatus* and *M. opacus*. It was not until these worms were sectioned that they were found to be of the same species as those taken from other turtles. Three worms from a fifth specimen of *Graptemys*, and those taken from specimens of *Chelydra* and *Chrysemys* (1946, Rausch, J. Parasitol. **32**) were similar, in general, to those described from fish hosts. None of the specimens from Ohio turtles has been spinose.

No North American species of this genus has previously been recorded from a warm-blooded host, although Ochi (1928, Tokyo Iji Shsh. 2578. Biol. Abs. **4**: 6043. 1930) records a species of this genus from mammalian hosts in Japan. Evidence is at present insufficient to justify describing the specimens from the raccoon as new, since it is impossible to determine whether the differences are specific, or have been brought about by development in an unusual host animal. It would be very desirable to attempt infection of different animals with metacercariae of *Microphallus ovatus* in order to gain more information concerning host specificity in this group.—Robert Rausch, *Dept. of Bacteriology and Public Health, Michigan State College, E. Lansing, Mich.*

THE AEROSOL BOMB AS A METHOD FOR DETERMINING THE TRIATOMIC INDEX IN HUMAN HABITATIONS IN CHAGAS' DISEASE ENDEMIC ZONES

The use of insecticides in the form of aerosols and the research of Sullivan, Goodhue and Fales (1942, J. Econ. Entom. **35**: 48–51) and Goodhue (1944, J. Econ. Entom. **37**: 338–341), which led to the development of the so-called "aerosol bomb" have stimulated investigations with the aim of discovering its possible further application against insects.

Following a suggestion of Professor Dr. Juan Noé, of the University of Santiago, the aerosol bomb has been tested against *Triatoma infestans*, known in Chile as "vinchuca" (a derivative from a quechua word meaning "the insect that drops himself upon us"), which lives in cracks and holes of the roofs and walls of houses in rural districts of North and Central Chile.

The aerosol bomb used by us was manufactured by the Pennsylvania Engineering Company and was supplied by Dr. R. Vicuña H., to whom sincere thanks are expressed. It contains pyrethrum, sesame oil and freon 12 (dichlorodifluoromethane). As a rule, a spray of 15–20 seconds per 40 cubic meters was applied. The experiments were carried out in rural houses near Santiago ("Las Condes," "El Salto").

The lethal action of the insecticide against *Triatoma infestans* was very low. Only 37 triatomids died out of 230 captured in 8 houses and the rest remained alive, until one month and a half later. It was not possible to determine whether the death of all the insects was due to the insecticide or to other causes. Nevertheless, during the experiments it was possible to observe an interesting fact: about five or ten minutes after spraying, large numbers of triatomids were observed creeping out from their hiding places, so that it became extremely easy to capture them. This happened even in habitations where it had previously not been possible to find either the triatomids or their feces. This effect was most apparent in rooms which were well sealed

before fumigation was undertaken and least satisfactory or slowest in those which were not closed at all. Table I summarizes the data collected.

TABLE I.—*Effect of pyrethrum aerosol spraying on triatomids*

House number	Cubic meters (approx.)	Spraying time (in seconds)	Number of triatomids observed or captured by 2 persons in 15 minutes	
			Before the spray	After the spray
1	45	15	0	81
2	40	20	2	17
3	36	10	1	32
4	42	15	1	7
5	48	20	2	14
6	30	10	3	60
7	50	20	0	3
8	45	20	3	16

In houses 3, 5 and 6 the spraying of aerosol was repeated after an interval of 10 minutes; twice several triatomids were captured on the outside of cracked walls. In some houses where the "aerosol test" was negative for triatomids, later application of dichloro diphenyl trichloroethane (DDT) in kerosene failed to reveal these insects crawling out of their day-time hiding places, such as occurred in the infested rooms.

Conclusion.—Based on the data obtained it may be concluded that the aerosol bomb containing pyrethrum should be useful in searching for triatomids inside human habitations and in determining the *triatomid index* when surveys on Chagas' disease are being carried out.

The writer expresses his thanks to Dr. E. C. Faust for assistance in the revision and preparation of the manuscript.—DR. AMADOR NEGHME, *Department of Parasitology, Public Health Service of Chile, Santiago, Chile.*

NOTE ON THE TICK, *IXODES ANGUSTUS* NEUMANN

The Rocky Mountain Laboratory of the United States Public Health Service at Hamilton, Montana, has three positive records of the tick *Ixodes angustus* Neumann biting humans: 12470, one female about one-third engorged, from the abdomen of a man, Newport, Oregon, June 6, 1931; 21073, one female, about two-thirds engorged, from a seven-year-old boy, Elma Washington, September 30, 1944; and 22029, one female about one-fourth engorged, from the scalp of a child, Clark County, Washington, October 3, 1945. In none of these instances was there any suggestion of disease transmission. *I. angustus* is a fairly common species which parasitizes a variety of small mammals. Published data and the records of the Rocky Mountain Laboratory show that it is particularly common in Washington, Oregon, and California but is only scatteringly present in other Western States as far south as New Mexico, in Alaska to about 60 degrees north latitude, and in British Columbia. There are a few records from Maine and Michigan, but none from the Central and Southeastern States.—R. A. COOLEY, *Senior Entomologist, U. S. Public Health Service.*

OBSERVATIONS ON *ANCYLOSTOMA* AND *TOXOCARA* INFECTION IN EXPERIMENTAL AND STOCK DOGS

The presence of parasites in experimental animals may interfere with and markedly influence the course or even alter the final result of an experiment. According to Scott (1930, Quart. Rev. Biol. 5: 79) anemia and oftentimes death occur in dogs with heavy hookworm infestation. It has also been observed here at Duke Hospital that dog hookworm caused death of a dog during nutritional studies (Susan G. Smith, personal communication). It became of interest, therefore, at the suggestion of Dr. A. S. Pearse of the Zoology Department of Duke University, to investigate the incidence of infection by the dog hookworm *Ancylostoma caninum* and the roundworm *Toxocara canis* among experimental and stock dogs housed in a common animal room in the Duke Hospital.

A. caninum ova were found in the feces of 19 of 24 dogs studied (79%). *T. canis* ova were present in the feces of 6 of 24 dogs (25%). Eighteen dogs were free of *T. canis* while 5 were free of both *A. caninum* and *T. canis*. The range of *A. caninum* infection in 19 dogs was from 1 to 2442 ova per gram of feces with a mean value of 413.4. The range of *T. canis* infection in 6 dogs was from 2 to 114 ova per gram of feces with a mean value of 52.2. It is concluded from this study that the incidence of infection with dog hookworm was high and roundworm relatively low when random sampling was taken of experimental and stock dogs housed in a common animal room. It is suggested that experimental laboratories make periodic surveys of their animals for possible nematode infestation and to eliminate this undesirable factor whenever discovered.—HENRY C. HILL, *Department of Zoology, Duke University, Durham, North Carolina.*

AMERICAN SOCIETY OF PARASITOLOGISTS

Advance Notice of Annual Meeting, Boston, Massachusetts, December, 1946

Because of difficulties and delays which now attend printing schedules and which may continue through 1946, it is deemed wise that plans for printing the program for the next meeting of the Society, to be held in Boston, Massachusetts, during the Christmas holidays, 1946, be formulated well in advance. The Society membership, therefore, is hereby advised that the deadline for the receipt of abstracts of papers for the Boston meeting will probably be September 1, 1946. The formal call for papers will be sent to members about August 1, 1946. It is hoped that the membership will bear in mind the early deadline date and will submit all abstracts in advance of that date, so that all papers can be included in the printed program.

Respectfully,

James T. Culbertson, *Secretary*

Mailing dates for Volume 31 (1945) were:

No. 1, April 6.
No. 2, June 6.
No. 3, July 19.
No. 4, September 5.
No. 5, November 20.
No. 6, (with December Supplement), February 26, 1946.

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